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A CORRELATION BETWEEN BACTERIAL ACTIVITY AND LIME REQUIREMENT OF SOILS

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INTRODUCTION

Limestone regions are noted for their fertility. Alfalfa, red clover, bluegrass, and corn are among the crops which thrive best on limestone soils. Those soils which do not naturally contain carbonate of lime are usually made more productive by applications of lime or limestone. Extensive investigations carried out by the Rhode Island, Maryland, Pennsylvania, Ohio, Illinois, and other agricultural experiment stations have demonstrated the value of lime in either the oxide, hydrate or carbonate form on soils which are acid to litmus. An excellent review of the most important investigational work on the use of lime on acid soils is given by Frear (9).

The investigations of Wheeler at the Rhode Island Agricultural Experiment Station, indicate, however, that a number of plants of economic importance thrive on soils which contain no solid carbonate of lime. Some of these plants are benefited by lime, but others are injured by applications of lime. Wheeler (36) says that orchard grass (*Dactylis glomerata*, L.) and meadow fescue (*Festuca elatior*, L.) are less injured by soil acidity than Kentucky blue-grass (*Poa pratensis*, L.) and timothy (*Phleum pratense*, L.) and that awnless brome grass (*Bromus inermus*, L), red top (*Agrostis alba var. vulgaris*, Thurb.), and Rhode Island bent (*Agrostis canina*, L.) do not seem to be susceptible to injury even on decidedly acid soils. He also states (37) that Concord grapes are apparently indifferent to the lack of lime and that cranberries, raspberries, and lima beans are injured by liming, the last named growing splendidly on soils so acid as to entirely destroy lettuce, spinach, onions, beets and asparagus. In his latest publication on this subject Wheeler (38) gives a summary of his work in which he shows that plants vary in their requirements from those which are injured by applications of lime even to a very acid soil, to those which are unable to live on an acid soil and are benefited remarkably by lime.

Coville (6) states that the blueberry, cranberry, strawberry, blackberry, red respberry, potato, sweet potato, rye, oats, millet, buckwheat, red top, carrot, turnip, cowpea, hairy vetch, crimson clover, soybean, lupine, and serradella are adapted to acid soils. He concludes, "soil acidity is not always

an objectionable condition which invariably requires lime" and "under certain conditions, a complete system of acid agriculture is practicable."

Harter (14) writes that liming has been shown to be beneficial to all crops on Norfolk soils with the exception of beans, peas, and tomatoes. Kossovitch and Althausen (26) report that, while the liming of acid podzol soils strikingly increases the yields, the limit of increase is at about the point of neutralization and that an excess injures the plants. No statement is made as to how the point of neutralization was determined. Heinrich (15) concludes that the determination of lime in a soil, by digesting with 10 per cent hydrochloric acid, can be used as an index in determining what crops will thrive. According to his report, the least amounts of lime which will permit of successful growth are:

Crops	*Calcium carbonate in the soil* per cent
Lupines, potatoes, and rye	0.05
Oats and barley	0.05 to 0.10
Peas and vetch	0.10
Red clover	0.10 to 0.12
Alfalfa	0.20 to 0.30

Fred and Graul (10) experimenting with alfalfa, soybeans, and red clover on acid soils of two series, conclude that half enough lime to neutralize the soil acidity as measured by the Truog (32) method is sufficient for the production of good yields of these crops on acid soils of these two series.

THE RELATION BETWEEN BACTERIAL ACTIVITY AND THE REACTION OF SOILS

One of the reasons usually given for the maintenance of a neutral or slightly alkaline reaction in soils is that the soil microörganisms, which have to do with the processes of decay and the changes by which certain organic and inorganic substances become available for higher plants are unable to work to best advantage in an acid medium. The ammonifying, nitrifying, and nitrogen-fixing bacteria are thought to prefer a neutral or slightly alkaline medium. However, it is probably true that the various groups of soil bacteria are differently affected by the soil reaction. The influence of acidity and alkalinity on the development of pathogenic bacteria has been studied by a number of investigators. The literature on this subject is reviewed quite fully by Itano (21). The degree of acidity or alkalinity which the organisms are able to withstand varies with the species. Certain forms, e.g., *Bacterium tuberculosis*, are able to live in the presence of a considerable degree of acidity. It is reasonable to believe that soil microorganisms show similar differences in this respect. The fact that many acid soils are supporting vegetation, indicates that bacterial processes are being carried on in them, although these processes might be materially hastened if lime were applied.

The number of bacterial colonies from soil aliquots which will develop on

agar plates is influenced by the reaction of the medium. Hoffmann (16) finds in counting the number of bacteria in soils that a medium slightly acid to phenolphthalein is more favorable than a medium which is neutral or slightly alkaline to phenolphthalein. Fischer (8), who conducted probably the most extensive investigations on the effect of lime on the number of bacteria in soils, shows that an application of either calcium oxide or calcium carbonate has a very marked effect in increasing the total number of bacteria.

That the rate of ammonification is increased by applications of lime is shown by Voorhees and Lipman (35). Coville (6) points out that many soils acid to litmus contain large amounts of ammonia. Kopeloff (25) shows that "where the soil reaction is unfavorable for the activities of the soil bacteria concerned in ammonification, the soil fungi may prove to be an important compensating factor."

The rate of nitrification is increased by applications of lime on soils which give an acid reaction with litmus. The results obtained by Lyon and Bizzell (27) are typical. A number of other investigators report similar effects from the use of lime. Scales (29), studying the activities of nitrifying organisms, finds they are most active in the presence of 50 per cent of the calcium-carbonate requirement (Veitch) of the soil. An excess of calcium carbonate seems to be toxic to the nitrifying organisms. Temple (31) finds that if an organic source of nitrogen is used instead of ammonium sulfate, the formation of nitrates is much greater in acid soils. He explains this increased nitrification on the basis of the formation of neutral zones, caused by the production of ammonia, at which points conditions are favorable for nitrification. Temple also shows that calcium salts of organic acids can be used as effectively as calcium carbonate in overcoming the toxic effect of ammonium sulfate on an acid soil. Miller (28), working with a sandy soil acid to litmus, finds that an application of 0.1 per cent of calcium oxide caused a decrease in the ability of the soil to nitrify ammonium sulfate and that 0.5 per cent of calcium oxide stopped the process entirely. Hutchinson (19) finds that calcium oxide acts not alone as a neutralizing agent, but also as a partial sterilizing agent. Since in the experimental work following applications of neutralizing agents are confined to calcium carbonate, it does not seem necessary to include any further discussion on the effect of calcium oxide on the bacterial processes in the soil.

It should be remembered that it has been shown that nitrate nitrogen is not necessary for all plants. Hall and Miller (12) call attention to the fact that ammonium sulfate, on the Park plats of the Rothamsted Farm, produces very good crops of grass, although the soil is deficient in lime and very little nitrification takes place. Hutchinson and Miller (20) find that peas are able to utilize ammonia nitrogen as well as nitrate nitrogen, although the opposite is true with wheat. Kelley (24) shows that rice, grown in swamp land, secures its nitrogen in the form of ammonia. If ammonification processes are less affected than nitrification processes by a deficiency of lime in

the soil, then plants which are able to utilize ammonia can survive where those depending on nitrate nitrogen cannot live.

Hopkins (18) notes that the application of lime increases the power of *Bacillus radicicola* in certain legumes to fix atmospheric nitrogen. Whiting (39) writes that nodules are often found in abundance on legumes on very acid soils. Japanese clover (*Lespedeza*) has often been observed by the writer growing on soils strongly acid to litmus and the roots were well supplied with nodules. These nodules were mostly near the surface of the soil. Kellerman and Robinson (22) find that crimson clover inoculation is little affected by the reaction of the soil. Fred and Graul (10) find that, if acid Colby silt loam soil is previously inoculated with *B. radicicola*, nitrogen fixation by soybeans is little influenced by applications of calcium carbonate. They also find this true on acid Colby silt loam with red clover. Both clover and alfalfa were able to fix considerable amounts of nitrogen when growing on Colby silt loam and Plainfield sand having only one-half of their acidity (Truog method) neutralized. The Colby silt loam required 10,400 and the Plainfield sand 5200 pounds of calcium carbonate to neutralize one-half of the acidity in 2,000,000 pounds of soil. Determinations of the lime requirement (Veitch) on the Colby silt loam soil, chosen from the same locality the year previous, showed a need of 3234 pounds of calcium carbonate per 2,000,000 pounds of soil. The authors state that "the Truog method shows much larger amounts of soil acidity than the Veitch."

Ashby (1) shows that the use of lime on the Rothamsted soils more than doubled the nitrogen-fixing power of the *Azotobacter*. Hoffman and Hammer (17) find that calcium carbonate is essential to non-symbiotic nitrogen fixation, but that the amount required is very minute and was present in sufficient amount in all the soils tested. These soils were chosen from various localities in Wisconsin and must have included some soils acid to litmus, since Whitson and Weir (40) estimate that two-thirds of the soils of Wisconsin are acid. Christensen and Larsen (4) find that if Ashby's solution is inoculalated with a soil in need of lime, the brownish film usually produced by *Azotobacter* does not develop. They suggest this as a method of determining the need of a soil for lime.

Gimingham (11) describes several organisms capable of bringing about the formation of carbonates from calcium salts of organic acids. Hall and Miller (13) also report that calcium salts of organic acids are transformed to the carbonate by soil organisms, the organic acids being decomposed to form carbon dioxide and water. Drew (7) shows that marine bacteria precipitate calcium carbonate from sea water. He names the organism responsible for this reaction, *Bacillus calcis*. Kellerman and Smith (23) write that it is possible in the laboratory to produce calcium carbonate by three types of biological processes; by the action of ammonium carbonate on calcium sulfate; by the action of ammonium hydroxide on calcium acid carbonate, and by the decomposition of calcium salts of organic acids. They state that Drew's organ-

ism is *Pseudomonas calcis*. This is a denitrifying organism which produces ammonia by the reduction of nitrates. Bear and Salter (2) show that the lime requirement (Veitch) of the West Virginia Agricultural Experiment Station fertility plots is less where the content of organic matter has been increased, and suggest that this decrease may have been due to the precipitation of calcium from solution by the humus in the soil, whereby it was prevented from being lost in the drainage water. This calcium might later be freed as the carbonate, as the decomposition of the organic matter was brought about by the soil organisms.

OBJECT OF THESE INVESTIGATIONS

In view of the fact that large areas of land are acid and that the distance from the supply of lime often makes the cost of applying large amounts of lime or limestone prohibitive, it was thought it might be desirable to consider more carefully the possibilities of a system of acid agriculture as suggested by Coville (6). Since the problem of the economy of nitrogen and its availability for the use of crops is largely a bacterial problem, it seemed important to study the relation of the reaction of the soil to the activities of the bacteria concerned in nitrogen accumulation and transformations. Recognizing the fact that plants do grow on soils which are acid to litmus, how are these plants supplied with nitrogen? We know that lime and limestone are valuable soil amendments, but might it not be possible that small applications of these materials would be relatively more effective in promoting the activities of the bacteria concerned in the nitrogen problem than large applications? If the *B. radicicola* of some legumes is more resistant to acidity than the *B. radicicola* growing on other legumes, might it not be possible to select legumes adapted to the reaction of the soil instead of adding lime to the soil to make the reaction suitable for the legumes we desire to grow? Even if nitrogen-fixing organisms are able to grow in acid soils, are they able to fix atmospheric nitrogen in such an environment? To answer these questions, it was proposed to measure the activities of those bacteria concerned in the nitrogen economy of plants as influenced by various amounts of calcium carbonate applied to acid soils.

DEFINITION OF "LIME REQUIREMENT"

In the preceding discussion, a rather loose construction is given to the term "soil acidity." This is simply in accordance with precedents set by the various investigators whose work is reviewed. As a rule, an "acid" soil means a soil which changes blue litmus paper red. The "degree of acidity" of soils has no such definite meaning, consequently the investigations reported are not strictly comparable. The writer sees no reason to disagree with Truog (33) as to what "soil acidity" really is. Truog writes that acid silicates are the main cause of soil acidity in upland soils. His excellent review of this subject gives a select bibliography of the investigational work along this

line. Truog (32) also writes that the acidity of soils may be conveniently divided into two classes, "active" and "latent" acidity. He states that "latent" acidity is undoubtedly much less injurious to plants than "active" acidity. He also shows the desirability of knowing the "avidity" of the active soil acids. Sharp and Hoagland (30) attempt to measure the lime requirement of soils by determining the hydrogen-ion concentration of the soil suspensions. The recent review of Clark and Lubs (5) of the literature on this subject, indicates that the hydrogen-ion concentration of the medium is the important factor to consider in the relationship between acidity and biological processes. The hydrogen-ion concentration of a soil in suspension in water is, however, not a measure of the amount of lime necessary to add to an acid soil to produce a neutral reaction of the soil. This is partly because of the slow solubility of the acid-forming constituents present in soils.

At the time this investigation was begun, most of the recent work on soil acidity had not been published. The writer felt at that time that the most satisfactory measure of the "lime requirement" of a soil was that obtained by the Veitch (34) method. Accordingly, this method was used in determining the quantitative need of the soils used for lime. It is interesting to note in this connection that when the two soils which were used most largely in these investigations had been treated with the quantity of calcium carbonate necessary to satisfy their lime requirements (Veitch) and had been mixed once each week for 12 weeks, they were found to be neutral to litmus paper.

HISTORY OF THE SOILS USED IN THESE EXPERIMENTS

A large part of the work reported has been done on samples of soil from two different localities belonging to different soil series. Both of these were acid in reaction, as will be shown later.

Soil I was secured from plot 18 of the West Virginia Agricultural Experiment Station farm. The soil is classified by the United States Bureau of Soils as Dekalb silt loam. It is a residual soil which has been formed by the disintegration of sandstone and greenish gray shales overlying the Pittsburg coal. The original timber was largely oak and chestnut with an occasional locust. The analysis of this soil is as follows:

Element	*Pounds per 2,000,000 of soil*
Nitrogen	1,940
Phosphorus	600
Potassium	25,100
Carbon	23,900
Calcium	2,300
Magnesium	4,300
Calcium carbonate requirement (Veitch)	3,500

Plot 18 has not received any fertilizer, lime or manure since the beginning of the fertilizer tests in 1900. Only a partial record of the produce of this

plot is available. During a part of the time since 1900 a tile drain, which passed near this plot, was not working, and, since the yields of the plot were somewhat abnormal, no permanent records of the plot were kept. Later the record of the produce of this plot was continued. This record shows that plot 18 corresponds normally in productivity to plot 21, which also received no fertilizer, lime or manure. The sample of soil was chosen from plot 18 because its record was incomplete and any change due to the removal of a large sample of soil would not interfere with the plot experiments. Since 1900 the following crops have been grown on these plots; rye, 1900 and 1907; wheat, 1901 and 1914; clover, 1902, 1909, and 1915; corn, 1903, 1905, and 1912; cowpeas, 1904; potatoes, 1906; timothy, 1909, 1910, and 1911, and oats, 1913. Table 1 gives the records of the fertilizer treatment and total produce of all the plots up to and including 1915.

TABLE 1

Total amounts of fertilizers applied and total produce per acre from 1900 to 1915 on soil I

PLOT	TREATMENT	NITRATE OF SODA	ACID PHOSPHATE	SULFATE OF POTASH	LIME (CaO)	MANURE	TOTAL PRODUCE
		pounds	*pounds*	*pounds*	*pounds*	*tons*	*pounds*
19	N, P, K, CaO.....	4200	4200	1625	4500		120,605
20	M, CaO.....				4500	210	152,400
21	Check.....						38,600
22	CaO.....				5500		36,615
23	Ash M, N.....	300	Ash of 40 tons of manure until 1912				39,270
24	Check.....						43,075
25	M.....					190	139,670
26	N, P, K.....	4200	4200	1625			117,910
27	Check.....						42,170
28	P, K.....		4200	1625			76,995
29	N.....	4200		1625			52,215
30	Check.....						39,480
31	N, P.....	4200	4200				95,940
32	K.....			1625			41,565
33	Check.....						36,845
34	P.....		4200				63,415
35	N.....	4200					41,195

N, indicates nitrate of soda; P, acid phosphate; K, sulfate of potash; M, manure.

Soil II was secured from the Ohio Agricultural Experiment Station farm at Wooster. This soil is classified by the Bureau of Soils as Wooster silt loam. It has been formed from the disintegration of sandstone and shales of the Mississippian period, under the influence of glacial action. The analysis of the soil used is as follows:

Element	*Pounds per 2,000,000 of soil*
Nitrogen	1,775
Phosphorus	664
Potassium	34,000
Carbon	22,200
Calcium	4,470
Magnesium	6,596
Calcium carbonate requirement (Veitch)	3,500

It will be observed that soil II has the same calcium-carbonate requirement as soil I.

Soil II has never received any fertilizer, lime or manure since the beginning of the fertilizer tests in 1893. Continuous records since that time have been kept on soil of the same history as this soil in a 5-year rotation experiment at the Wooster station. The rotation has been corn, oats, wheat, clover, and timothy. A summary of the effect of lime and fertilizers on this soil is given by Williams (41) in table 2. An experiment has also been in progress on this same type of soil which had been kept in a fair state of fertility by a good rotation and an occasional application of manure previous to the beginning of the experiment. The rotation since practiced has been corn, oats, and clover. The records of this experiment are shown in table 3. It will be seen by a study of tables 2 and 3, that both lime and acid phosphate are very effective in increasing the yields of the crops grown in these two rotations. While lime is very efficient, it seems remarkable that such large yields of these crops can be produced by the use of acid phosphate alone on a soil which has a calcium-carbonate requirement of 3500 pounds per 2,000,000 pounds of soil.

The other samples of soil used in these experiments were Dekalb soils chosen from various localities in West Virginia. These soils vary greatly because of differences in the systems of management they have undergone. Analyses of these soils are shown in subsequent tables.

PLAN OF THESE EXPERIMENTS

Large samples of soils, acid to litmus, were secured, sent immediately to the laboratory, made to pass a 2-mm. sieve, and stored in large galvanized iron cans. From these cans soil was removed as needed. Careful analyses of the soils were made for the total amount of nitrogen, phosphorus, potassium, calcium, magnesium, and carbon. Lime-requirement determinations were made by the Veitch method as indicated above. Amounts of C. P. calcium carbonate varying from 250 pounds to 40,000 pounds per 2,000,000 pounds of soil were added to the soils. A study was made of the effects of these applications on: (a) the number of bacteria, (b) the rate of ammonification, (c) the rate of nitrification, (d) the fixation of nitrogen by non-symbiotic organisms, and (e) the development of *B. radicicola* of the soybean. All analyses were made according to the methods given by Bear and Salter (3).

The calcium carbonate was applied and mixed thoroughly with the soil, which was then placed in 1-gallon stone jars. Enough water was added to the soil to give it an optimum moisture content. Each week the soil was removed from the jars and mixed thoroughly and the loss of moisture, due to

TABLE 2

The effect of lime on the yields of crops on soil II

PLOT	TREATMENT	YIELD PER ACRE									
		Corn 1900–1915		Oats 1901–1916		Wheat 1906–1916		Clover 1903–1916		Timothy 1909–1916	
		Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed
		bus.	*bus.*	*bus.*	*bus.*	*bus.*	*bus.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
2	Phosphorus*	35.51	42.32	39.16	42.85	21.48	25.17	1848	2680	3058	3810
8	Phosphorus,* potassium	43.95	51.08	42.62	46.38	22.17	26.38	2144	3166	3125	3881
11	Phosphorus*, potassium, nitrogen	47.67	55.12	49.77	49.71	31.27	31.86	2683	3388	3445	4124
17	All three with less nitrogen but more phosphorus*	47.23	55.67	51.84	52.38	27.32	30.85	2492	3598	3364	4543
18	Barnyard manure	56.31	61.68	43.62	44.93	29.51	32.49	3448	4393	4525	5531
24	Same as 17 but nitrogen in sulfate of ammonia	46.23	55.98	48.21	51.36	24.70	31.26	2139	3544	3111	4409
26	Same as 17 but phosphorus in bone meal	46.01	51.17	46.37	46.81	27.78	28.65	2945	3772	3504	4585
29	Same as 17 but phosphorus in basic slag	46.27	51.69	47.77	47.85	29.76	28.93	2981	3371	3741	4306
Average unfertilized		26.48	32.32	27.19	32.08	12.74	16.09	1276	1841	2500	3069

* Phosphorus in the form of acid phosphate.

TABLE 3

The effect of lime and acid phosphate on soil II

TREATMENT	AMOUNT PER ACRE	CORN 9 YEARS		OATS 9 YEARS		CLOVER 8 YEARS
		Grain	Stover	Grain	Straw	Hay
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
No fertilizer		51.50	2759	44.94	1961	4074
Calcium oxide	1000	57.33	3149	47.53	2079	4580
Ground limestone	1780	54.84	2820	45.35	1876	4362
Acid phosphate	320	60.18	3056	46.16	1912	4277

evaporation, was restored. This was continued for 12 weeks in order that the soil microörganisms should have time to adjust themselves to the changes in soil reaction. At the end of that time, the determinations of nitrifying power, ammonifying power, etc., were made. These determinations required about one-half of the soil.

Since the analyses showed that these soils were very deficient in total phosphorus, a thing which is commonly true of acid soils, it seemed advisable to apply phosphorus in a readily available form in order to remove it from being a possible limiting factor in the various bacterial activities studied. Accordingly, 0.2 per cent of mono-calcium phosphate, equivalent to 1000 pounds of phosphorus per 2,000,000 pounds of soil, was added, the moisture content was again restored, and the mixing was continued for another period of 12 weeks. At the end of this time, the above determinations were repeated. In some of the later experiments the calcium carbonate was added just previous to the time of studying the rate of nitrification, ammonification, etc.

THE EFFECT OF CALCIUM CARBONATE ON THE NUMBER OF BACTERIA

Soils I and II were used in these experiments, after they had received the various applications of calcium carbonate and had been mixed thoroughly each week for 12 weeks, as previously outlined. Plate counts of the number of microörganisms were made at the end of the 12-week period. After the 0.2 per cent of mono-calcium phosphate had been added and mixed with the remainder of the soil each week for a second 12 weeks, plate counts were again made. Aliquots of the soil suspension were plated on Heyden agar. The plates were incubated at room temperature and counts were made at the end of 6 days. Table 4 shows the results of these counts. Each figure represents the average of four plates.

As might be expected, the greatest relative change in the number of bacteria occurred after the neutral point had been passed. This was true in

TABLE 4

The effect of calcium carbonate on the number of bacteria

CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	BACTERIA PER GRAM OF SOIL			
	Soil I without phosphorus	Soil I with phosphorus	Soil II without phosphorus	Soil II with phosphorus
pounds				
0	3,341,000	4,150,000	3,503,000	3,438,000
250	4,127,000	4,320,000	3,418,000	3,536,000
500	3,537,000	3,540,000	4,614,000	4,421,000
1,000	3,439,000	2,750,000	3,781,000	5,207,000
2,000	3,930,000	2,520,000	4,472,000	5,781,000
3,000	4,127,000	4,090,000	4,919,000	5,683,000
Neutral point (Veitch method)				
4,000	4,422,000	5,820,000	7,348,000	10,005,000
5,000	5,306,000	7,700,000	9,741,000	17,392,000
7,500	5,601,000	7,070,000	15,827,000	14,297,000
10,000	3,341,000	6,680,000	14,973,000	7,959,000
20,000	6,682,000	10,750,000	14,892,000	3,635,000
40,000	9,335,000	13,050,000	18,199,000	9,826,000

both soils, as shown in figure 1. The 4000 and 5000-pound applications of calcium carbonate resulted in relatively large increases in numbers. Additions of calcium carbonate in excess of 7500 pounds per 2,000,000 pounds of soil gave somewhat uncertain results. There was a decrease in every case accompanying an application of 10,000 pounds as compared with 7500 pounds of calcium carbonate. The 20,000 and 40,000-pound applications brought about marked increases in numbers. These fluctuations were probably due to the adjustment of the soil reaction to the point where it was more suitable to the requirements of some forms which developed in vast numbers under this optimum soil reaction. It seems quite evident that the application of calcium carbonate caused decided changes in the number of bacteria in these soils. The maximum increases in numbers had apparently not been reached in three of the four cases by applications of 40,000 pounds of calcium carbonate per 2,000,000 pounds of soil. Similar trials with calcium oxide produced marked decreases in the number of bacteria in these soils following the larger applications. This was probably due to the partial sterilizing action of calcium oxide previously referred to.

THE EFFECT OF CALCIUM CARBONATE ON THE RATE OF AMMONIFICATION

Soils I and II, after the treatments with calcium carbonate and monocalcium phosphate previously referred to, were used in the experiments on ammonification. The source of nitrogen was Hammarsten's casein. Enough casein was added to supply 160 mgm. of nitrogen per 100 gm. of soil. The soil was then given an optimum moisture content and incubated in tumblers for 3 days at room temperature, after which analyses were made for ammonia by distillation with magnesium oxide. Each figure given in table 5 represents the average of two determinations which checked usually within less than 1 mgm. per 100 gm. of soil. Other determinations, not reported in this paper, were made in which only 40 mgm. of nitrogen were added, with very satisfactory results. The author believes that 160 mgm. of nitrogen per 100 gm. of soil are likely to produce abnormal conditions in a soil, although in most of the ammonification experiments reported in the literature even larger amounts of nitrogen were supplied.

The greatest relative increase in the rate of ammonification of casein, per unit of calcium carbonate applied, occurred with applications of 2000 pounds of calcium carbonate per 2,000,000 pounds of soil, as shown in figure 2. There was no marked increase in ammonification as the neutral point was passed. As previously shown, this was also the case in the number of bacteria. Applications of 250 pounds of calcium carbonate per 2,000,000 pounds of soil had a tendency to cause a decrease in the rate of ammonification. Applications of calcium carbonate in excess of 5000 pounds caused only a slight increase in the amount of ammonia produced. The 20,000 and 40,000-pound applications caused slight decreases in ammonia in several cases. There was

apparently no definite correlation between the number of bacteria and the amount of ammonia produced, although in general, increased amounts of calcium carbonate resulted in larger numbers of bacteria and more rapid ammonification.

THE EFFECT OF CALCIUM CARBONATE ON THE RATE OF NITRIFICATION

The effect of calcium carbonate on the rate of nitrification in soils I and II is shown in table 6. All figures in this and in succeeding tables of nitrification

TABLE 5

*The effect of calcium carbonate on the rate of ammonification of casein**

CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NITROGEN AS AMMONIA PER 100 GM. OF SOIL					
	Soil I without phosphorus	Soil I with phosphorus	Soil II† without phosphorus	Soil II without phosphorus	Soil II with phosphorus	Soil II with phosphorus
pounds	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0	72.40	60.70	38.85	22.89	29.51	39.20
250	71.00	61.00	40.25	22.05	25.55	37.38
500	72.80	59.60	45.36	29.05	28.00	37.80
1,000	75.40	62.40	48.02	29.40	28.63	44.31
2,000	78.50	68.00	57.54	41.65	43.40	55.44
3,000	79.00	70.00	56.00	44.59	45.36	57.75
Neutral point (Veitch method)						
4,000	78.40	70.60	60.41	45.85	56.84	63.00
5,000	76.30	70.80	67.27	57.12	64.61	71.54
7,500	85.30	74.80	71.40	57.05	52.29	67.97
10,000	83.60	74.80	74.48	62.79	63.07	71.61
20,000	85.40	77.80	77.40	60.76	55.27	68.81
40,000	87.50	77.60	77.42	61.25	51.61	59.36

* The results in each vertical column were obtained on the same day. Fluctuations in the temperature in the room are responsible for some of the differences observed in horizontal columns.

† Four-day periods of incubation.

represent averages of two determinations. As a rule, the duplicates agreed within less than 0.1 mgm. per 100 gm. of soil. Accordingly, only averages are reported.

These soils were treated with calcium carbonate in varying amounts and with mono-calcium phosphate as previously outlined. At the end of the 12-week periods, samples of these soils of 100 gm. each were placed in 1000-cc. Erlenmeyer flasks for the nitrification experiments. To each flask were added 20 mgm. of nitrogen in the form of either ammonium sulfate or ammonium carbonate. After adding water to the optimum content, the soils were incubated for 21 days at room temperature, after which the nitrate determinations were made by the phenol-disulphonic acid method.

A study of table 6 and figure 3 shows that the addition of calcium carbonate is followed by an increased nitrification which correlates almost directly with the increased application of calcium carbonate. This correlation holds fairly well in every case with applications up to 5000 pounds per 2,000,000 pounds of soil. There is no sudden break in the correlation as the neutral point is passed. Applications of calcium carbonate in excess of 5000 pounds are followed by increased nitrification, although the curve of increase begins to incline more toward the horizontal. In half of the experiments the curve was still ascending with applications of 40,000 pounds of calcium carbonate.

TABLE 6

The effect of calcium carbonate on the rate of nitrification

CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NITROGEN AS NITRATE PER 100 GM. OF SOIL							
	Source of nitrogen, ammonium sulfate				Source of nitrogen, ammonium carbonate.			
	Soil I* without phosphorus	Soil I with phosphorus	Soil II without phosphorus	Soil II with phosphorus	Soil I without phosphorus	Soil I with phosphorus	Soil II without phosphorus	Soil II with phosphorus
pounds	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0	1.08	4.06	5.28	6.07	1.38	7.22	5.29	7.50
250	1.38	4.32	4.39	5.34	1.93	8.24	5.00	8.00
500	1.43	4.60	4.40	6.38	2.11	8.42	5.42	8.40
1,000	1.82	5.24	6.15	6.75	2.21	9.52	6.60	8.55
2,000	2.29	6.38	8.50	8.73	3.01	12.42	9.03	11.55
3,000	2.96	9.34	10.48	10.43	3.55	15.30	10.04	12.50
Neutral point (Veitch method)								
4,000	3.13	11.92	15.74	12.50	3.28	17.50	11.87	15.12
5,000	3.44	13.86	15.96	15.00	4.11	18.00	15.77	18.75
7,500	3.48	16.37	18.18	15.38	4.69	19.00	17.27	16.35
10,000	4.44	19.35	20.98	15.98	4.30	20.00	20.30	16.16
20,000	4.00	20.45	22.87	16.00	4.32	20.96	19.80	16.00
40,000	4.20	22.55	19.88	15.00	5.18	23.30	20.57	15.00

* An error was made in calculating the optimum moisture content and the soil in this experiment was too dry.

This is directly contrary to the work of Scales previously referred to, which indicated that 50 per cent of the amount of calcium carbonate necessary to supply the lime requirement of the soil is sufficient to attain the maximum rate of nitrification. Additional amounts are reported to have acted injuriously.

The marked increase in the rate of ammonification observed with the addition of 2000 pounds as compared to 1000 pounds of calcium carbonate per 2,000,000 pounds of soil was not followed by a coresponding increase in the nitrification. No correlation was found between the increased number of bacteria in the soil and the rate of nitrification except that in general the

application of increased amounts of calcium carbonate caused an upward tendency in the number of bacteria, as well as in the rate of nitrification. Since the agar plate method is not designed to include the nitrifying bacteria, no data are available as to the actual number of nitrifying organisms which were present in the soils following the applications of varying amounts of calcium carbonate.

EFFECT OF CALCIUM CARBONATE ON THE RATE OF NITROGEN FIXATION BY NON-SYMBIOTIC SOIL ORGANISMS

Samples I and II were employed again in these experiments after they had been treated as previously described. Shallow dishes having a depth of about 3 inches and a capacity of 400 gm. of soil were used for this work. Soil

TABLE 7

The effect of calcium carbonate on nitrogen fixation by non-symbiotic soil organisms

CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NITROGEN FIXED PER 100 GM. OF SOIL		
	Soil I without phosphorus	Soil II without phosphorus	Soil II with phosphorus
pounds	*mgm.*	*mgm.*	*mgm.*
0	0.7	0.3	2.0
250	0.3	0.8	3.6
500	0.5	0.8	3.6
1,000	1.0	0.6	4.6
2,000	0.7	1.4	3.8
3,000	0.5	2.3	11.3
Neutral point (Veitch method)			
4,000	0.1	2.8	12.2
5,000	1.8	4.1	12.7
7,500	2.0	6.0	15.8
10,000	1.5	5.8	10.1
20,000	2.1	4.5	12.6
40,000	1.4	4.4	9.9

from the various pots to which the calcium carbonate and mono-calcium phosphate had been applied was placed in the dishes and mixed thoroughly with 2 per cent of mannit. Optimum moisture conditions were secured and maintained as nearly as possible by adding water twice daily to restore that lost by evaporation. The soils were incubated 21 days at room temperature. After thorough drying, the entire samples were pulverized to pass a 100-mesh sieve and the total nitrogen was determined in triplicate. The triplicates agreed usually within 0.05 mgm. of nitrogen on 10-gm. samples.

From a study of table 7, it appears evident that both calcium carbonate and mono-calcium phosphate were essential to the highest fixation of nitrogen. The mono-calcium phosphate had such a marked effect in increasing the nitrogen-fixing power of soil II, as shown in figure 4, that it would seem that phos-

phorus was equally as important as lime for the nitrogen-fixing organisms in this soil. The largest relative increase in nitrogen fixation followed an application of 3000 pounds of calcium carbonate per 2,000,000 of soil when accompanied by the use of mono-calcium phosphate. Heavier applications of calcium carbonate caused an increase in nitrogen fixation until as much as 10,000 pounds per 2,000,000 pounds of soil had been applied. This amount and heavier applications caused a decrease in nitrogen fixation. Apparently phosphorus was a limiting factor in nitrogen fixation in soil I, although time did not permit an experimental test of this point. The good effects resulting from the use of acid phosphate on these soils under field conditions may be due in part to this increased nitrogen fixation accompanying its use. This again is indicated by the analyses of the West Virginia Station fertility plots from which the author and others have shown that the plot receiving acid phosphate and sulfate of potash has accumulated 1173 pounds of nitrogen per acre during the last 15 years which could not be accounted for except by nitrogen fixation from the air. The evidence shown in table 7 indicates that calcium carbonate is necessary in addition to the phosphorus for the most effective nitrogen fixation.

Following the suggestion of Christensen and Larsen (4), soil from each of the pots to which varying amounts of calcium carbonate had been applied, was used to inoculate Ashby's solution in order to study the relation between the film development on the surface of the liquid and the lime requirement of the soil. The brownish film was very well developed in the flasks inoculated with soil which contained an amount of calcium carbonate in excess of the requirement of the soil and practically disappeared as the quantity of calcium carbonate applied was reduced below the amount necessary to satisfy the requirement of the soil. The development of brown pigment is apparently closely related to the amount of lime in the soil and may be used as an index of the need of lime by the soil. Other experiments which will be discussed later indicated, however, that nitrogen fixation in Ashby's solution may take place when the solution is inoculated with soils having calcium-carbonate requirements as high as 4600 pounds per 2,000,000 pounds of soil. Apparently, the lack of development of the brownish film is not accompanied by the loss of ability to fix nitrogen, since no soil was found which did not show nitrogen fixation when inoculated into Ashby's solution and allowed to stand for 21 days at room temperature.

THE EFFECT OF CALCIUM CARBONATE ON THE FIXATION OF NITROGEN BY B. RADICICOLA OF THE SOYBEAN (SOJA MAX PIPER)

Soils I and II were again used in these experiments. One-gallon pots were filled with these soils and the calcium carbonate was added. Each pot received an application of 0.2 per cent of mono-calcium phosphate. The pots were planted to soybeans, the beans having been previously inoculated with

B. radicicola. Six beans were planted in each pot and later thinned to three per pot. After the beans had reached the stage where pods were formed, they were harvested, and records were taken of their green and dry weight, the number of nodules, the dry weight of nodules and the milligrams of nitrogen in the roots, tops and nodules. One crop was harvested from each pot during the summer of 1915 and another crop during the summer of 1916. The records are shown in tables 8 and 9.

The number of nodules had a tendency to increase slightly with small applications of calcium carbonate. Applications of more than 3000 pounds of calcium carbonate per 2,000,000 of soil caused a decrease in the number of nodules. This decrease was proportional to the amount of calcium carbonate applied. The dry weight of nodules was also decreased with large applications of calcium carbonate. The rate of decrease in dry weight of nodules with increased amounts of calcium carbonate was more marked than the rate of decrease in the number of nodules. The amount of nitrogen in the nodules was almost directly correlated with the dry weight of nodules, and decreased with additional quantities of calcium carbonate. It will be noticed that in both cases the dry weight and total nitrogen of both stems and roots had a tendency to increase with small applications of calcium carbonate, but that applications in excess of 2000 pounds per 2,000,000 of soil had a tendency to cause a decrease in dry weight and total nitrogen of the stems and roots.

The total nitrogen fixed by soils I and II during the two years in which the two crops of soybeans were grown was determined. Analyses of the soil were made before and after the beans were grown. The difference in the nitrogen content of the soil at these two periods plus the nitrogen removed in the nodules, stems and roots, after subtracting the nitrogen content of the seed and water used in watering the plants, represents the nitrogen secured from the air.

The total nitrogen fixed in two years per 2,000,000 pounds of soil, as shown in the last columns of tables 8 and 9, indicate that soil II has had a more active nitrogen-fixing flora than soil I. In so far as the chemical composition is concerned, the two soils correspond fairly well, as will be found by referring to the analyses of these two soils previously shown. By referring again to table 7, showing the rate of nitrogen fixation by *Azotobacter*, it will be seen that soil II was much more active in this respect than soil I. It is possible that a greater part of the nitrogen accumulated in soil II during the growing of the legumes was fixed in the soil through the agency of the non-symbiotic organisms. The nitrogen fixation had a tendency to decrease with applications of calcium carbonate in excess of 2000 pounds per 2,000,000 pounds of soil, although the lime requirement of both soils indicated a need of 3500 pounds. Apparently, with increased applications of calcium carbonate the rate of nitrification was so high, as indicated in table 6, that the soybeans were able to secure a greater part of their nitrogen in the form of nitrates. Large numbers of *B. radicicola* were present in all the pots whether treated with

TABLE 8

The effect of calcium carbonate on nitrogen fixation by B. radicicola of the soybean in soil I

POT	CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NUMBER	NODULES		STEMS		ROOTS		SOIL		
			Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Nitrogen in beginning per pot	Nitrogen at end per pot	Nitrogen fixed per 2,000,000 pounds of soil
	pounds		*mgm.*	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*	*grams*	*grams*	*pounds*
1	0	113	653	31	13.2	348	4.3	40	3.0009	2.8985	93
2	250	67	887	39	14.2	377	5.1	42	3.0039	2.8675	98
3	500	88	749	33	14.3	368	4.4	39	3.0039	2.8985	107
5	2,000	100	1017	47	17.3	464	3.7	35	3.0039	2.8272	130
6	3,000	72	560	27	14.9	303	3.4	38	3.0039	2.7900	+8
	Neutral point (Veitch method)										
7	4,000	65	317	16	11.9	342	2.9	37	3.0039	2.7683	−6
8	5,000	79	537	26	14.1	363	3.6	39	3.0039	2.8923	94
9	7,500	84	464	22	13.9	426	3.8	46	3.0039	2.7218	23
10	10,000	66	212	11	11.2	342	3.6	52	3.0039	2.6660	−60
11	20,000	45	220	13	10.3	298	5.1	58	3.0039	2.6815	−87
12	40,000	57	221	13	12.2	383	3.4	50	3.0039	2.6505	−56

0.1727 gm. of nitrogen in the soybeans planted.
0.0043 gm. of nitrogen in the water used in watering the soybeans.

TABLE 9

The effect of calcium carbonate on nitrogen fixation by B. radicicola of the soybean in soil II

POT	CALCIUM CARBONATE PER 2,000,000 POUNDS OF SOIL	NUMBER	NODULES		STEMS		ROOTS		SOIL		
			Dry weight	Total nitrogen	Dry weight	Total nitrogen	Dry weight	Total nitrogen	Nitrogen in beginning per pot	Nitrogen at end per pot	Nitrogen fixed per 2,000,000 pounds of soil
	pounds		*mgm.*	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*	*grams*	*grams*	*pounds*
1	0	79	781	38	14.8	394	4.1	46	2.6181	3.2008	570
3	500	75	744	37	15.2	440	4.0	42	2.6181	3.1860	587
6	3,000	148	577	29	13.5	371	4.7	45	2.6181	3.1418	511
	Neutral point (Veitch method)										
8	5,000	71	330	18	12.3	338	3.2	41	2.6181	3.1358	476
9	7,500	85	251	13	12.7	370	3.8	45	2.6181	3.1270	490
10	10,000	66	207	12	11.8	335	3.3	57	2.6181	3.0238	408
11	20,000	54	124	5	11.5	341	4.0	68	2.6181	2.9648	377
12	40,000	33	126	7	14.4	393	3.0	40	2.6181	2.9205	265

0.1727 gm. of nitrogen in the soybeans planted.
0.0043 gm. of nitrogen in the water used in watering the soybeans.

calcium carbonate or not, although quantitative determinations were not made of their numbers.

THE EFFECT OF CALCIUM CARBONATE ON SOYBEANS UNDER FIELD CONDITIONS

In order to determine whether soybean yields are increased by the use of calcium carbonate on soil I under field conditions, it was decided to grow soybeans on the fertility plots of the station farm during the summer of 1916. Three varieties of soybeans were sown in rows across the plots and cultivated during the growing season. One-half of each plot received an application of calcium carbonate at the rate of 2 tons per acre in the form of ground limestone. The yields of hay produced are given in table 10. The previous crop records and the analyses of the soils of these plots are given in tables 1 and 11.

TABLE 10

Effect of ground limestone on yield of soybean hay on soil I

PLOTS	TREATMENT	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS	YIELD OF HAY PER ACRE		INCREASE WITH LIMESTONE
			No limestone	Limestone	
		pounds	*pounds*	*pounds*	*per cent*
19	N, P, K, CaO	0	5270	5400	+2
20	M, CaO	0	6390	6850	+7
21	Check	2800	1605	1400	−13
22	CaO	0	1920	2430	+26
26	M	2800	7150	7360	+3
26	N, P, K	3200	5300	5370	+1
28	P, K	3600	2820	4690	+66
29	N, K	3400	1285	2280	+77
31	N, P	3200	3375	4460	+32
32	K	3600	1495	1995	+34
34	P	3400	3285	4105	+25
35	N	3400	1220	1705	+40
Averages			3426	4004	+17

From a study of the plots and the crop records, it would seem that the use of 2 tons of limestone per acre did not give sufficient increase in yield to justify the conclusion that soybeans will not grow well except on soils which have had their lime requirement satisfied. On plots 25 and 26, the soils of both of which have rather high calcium-carbonate requirements, but which also contain a fairly high content of nitrogen, the yield of soybeans was little affected by the limestone. This might mean that more nitrogen was secured from the soil on these plots and for this reason the crop was larger. However, the nodules were plentiful on the roots of the soybeans on plots 25 and 26 and, therefore, we could assume that nitrogen fixation from the air was taking place.

EFFECT OF CALCIUM CARBONATE ON THE BACTERIAL ACTIVITIES OF DEKALB SOILS HAVING VARYING LIME REQUIREMENTS

A large number of samples of acid soils all belonging to the Dekalb series were chosen from various parts of West Virginia and sent to the laboratory. From this number 12 samples were chosen which had calcium-carbonate requirements varying from 400 to 4600 pounds per 2,000,000 pounds of soil. The analyses of these soils are shown in table 11. These soils differ mostly because of the different systems of management practiced by the men who have farmed them since the areas from which the samples were chosen were cleared from the forest. Many of these areas had been farmed for from seventy-five to one-hundred years and others had not been farmed for more than a few years.

TABLE 11

Analyses of soils of table 12

SAMPLE	POUNDS PER 2,000,000 POUNDS OF SOIL			
	Nitrogen	Phosphorus	Carbon	Calcium-carbonate requirement
	pounds	*pounds*	*pounds*	*pounds*
III	3870	1203	41,420	400
IV	1904	586	21,790	1000
V	1669	680	17,600	1200
VI	3374	697	47,230	1400
VII	3142	902	32,140	1600
VIII	2042	1216	20,280	2000
IX	2602	662	32,450	2200
X	4142	1135	48,680	2600
XI	3384	660	39,490	2800
XII	2750	706	32,140	3200
XIII	1960	608	21,900	3800
XIV	3124	753	48,280	4600

The rates of nitrification, ammonification, and nitrogen fixation were studied in an attempt to determine whether there was any relation between the activities of the soil organisms and the calcium-carbonate requirements of these soils.

In nitrification studies 100 gm. of soil to which varying amounts of calcium carbonate had been added were placed in 1000-cc. Erlenmeyer flasks and incubated with optimum moisture content at room temperature for 21 days, using ammonium sulfate as the source of nitrogen, adding a sufficient amount to supply 20 mgm. of nitrogen per 100 gm. of soil. In ammonification studies 100-gm. samples of soil were used and varying amounts of calcium carbonate were added as in the nitrification tests. Casein was used as the source of nitrogen, 160 mgm. of nitrogen being added to 100 gm. of soil. The soil was incubated in tumblers at optimum moisture content for 3 days and the am-

monia determined by distillation with magnesium oxide. Nitrogen-fixation tests were carried on by placing 10 gm. of soil in 100 cc. of Ashby's solution in 800-cc. Kjeldahl flasks for a period of 21 days at room temperature. To one set of flasks enough calcium-carbonate was added to be equivalent to 10,000 pounds per 2,000,000 pounds of soil. At the end of 21 days the total nitrogen was determined. All of the determinations on nitrification, ammonification, and nitrogen fixation were performed in duplicate and these duplicates as a rule checked very closely. The results of these experiments are tabulated in table 12.

In general the highest rates of ammonification occurred with soils having the lowest calcium-carbonate requirements. The applications of 2000 pounds and 5000 pounds of calcium-carbonate brought about marked increased in the rate of ammonification. Applications of 10,000 pounds of calcium carbonate per 2,000,000 pounds of soil caused a decreased ammonification except in soils XIII and XIV, which had calcium-carbonate requirements of 3800 and 4600 pounds, respectively. Apparently the application of 10,000 pounds of calcium carbonate per 2,000,000 of soil on soils having calcium-carbonate requirements of less than 3800 pounds is injurious to ammonifying organisms.

There was no very definite correlation between the rate of nitrification of ammonium sulfate and the calcium-carbonate requirement of the soils. In general, the soils having high calcium-carbonate requirements had a very low nitrifying power. With soils having calcium-carbonate requirements in excess of 2200 pounds per 2,000,000 pounds of soil, the nitrifying organisms did not become markedly active even with large applications of calcium carbonate. Either the nitrifying organisms were almost entirely absent or had become very inactive because of the unfavorableness of the medium in which they were living.

There was no very marked correlation between the calcium-carbonate requirement of these soils and the nitrogen-fixing power of the soil organisms in Ashby's solution. Soil XIV, having a calcium-carbonate requirement of 4600 pounds, was able to fix nitrogen to the extent of 2.9 mgm. per 100 cc. of Ashby's solution in 21 days. The rate of nitrogen fixation was increased in every case by the addition of calcium carbonate, but the effect was more marked on soils having a high requirement than in soils having a low calcium-carbonate requirement. It seems remarkable, however, that nitrogen fixation took place in all cases even though some of the soils had very high lime requirements.

EFFECT OF FERTILIZERS ON THE BACTERIAL ACTIVITIES OF SOILS

These experiments were conducted in order to determine what effect differences in the fertilizer treatments of the same soil would have on the bacterial activities in the soil. Samples of soil were chosen from 12 plots of the fertilizer series of the fertility plots on the West Virginia station, some of which differ considerably because of the fertilizer applications they have received during the last 15 years. Records of the treatments of the soil on these plots have

TABLE 12

The effect of calcium carbonate on the activities of soil bacteria in Dekalb soils having varying lime requirements

SOIL	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS OF SOIL	CALCIUM CARCONATE APPLIED PER 2,000,000 POUNDS OF SOIL	NITROGEN PER 100 GM. OF SOIL				NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION	
			Nitrogen as ammonia from casein		Nitrogen as nitrates from ammonium sulfate			
	pounds	*pounds*	*mgm.*	*relative*	*mgm.*	*relative*	*mgm.*	*relative*
III	400	0	68.9	100	8.0	100	7.6	100
		2,000	76.5	111	12.6	158		
		5,000	87.6	127	12.5	156		
		10,000	82.4	118	9.4	118	8.7	114
IV	1,000	0	67.1	100	4.4	100	4.2	100
		2,000	81.2	121	8.4	191		
		5,000	86.4	129	13.5	307		
		10,000	74.6	111	14.0	318	5.6	133
V	1,200	0	68.9	100	1.3	100	2.7	100
		2,000	80.5	117	7.8	600		
		5,000	78.1	114	12.0	969		
		10,000	72.6	105	16.0	1231	4.1	152
VI	1,400	0	73.5	100	5.8	100	4.4	100
		2,000	83.2	113	7.3	126		
		5,000	86.9	118	8.0	138		
		10,000	87.7	119	12.6	217	5.6	127
VII	1,600	0	56.8	100	1.5	100	4.9	100
		2,000	77.3	136	3.0	200		
		5,000	91.8	161	5.7	380		
		10,000	87.5	154	16.0	1067	5.9	120
VIII	1,800	0	67.0	100	0.4	100	5.1	100
		2,000	76.2	114	1.5	375		
		5,000	96.6	129	8.0	2000		
		10,000	87.3	130	15.7	3925	6.5	127
IX	2,200	0	69.6	100	0.8	100	3.1	100
		2,000	83.1	119	5.1	638		
		5,000	90.1	129	9.8	1225		
		10,000	82.7	119	4.6	575	4.7	151
X	2,600	0	62.9	100	2.7	100	3.4	100
		2,000	75.1	119	4.8	144		
		5,000	92.2	147	5.2	156		
		10,000	91.8	146	13.0	390	4.8	141
XI	2,800	0	51.6	100	0.8	100	2.3	100
		2,000	68.3	132	2.2	275		
		5,000	88.9	172	1.7	213		
		10,000	92.2	159	2.1	263	3.2	139

TABLE 12—(Continued)

SOIL	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS OF SOIL	CALCIUM CARBONATE APPLIED PER 2,000,000 POUNDS OF SOIL	NITROGEN PER 100 GM. OF SOIL				NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION	
			Nitrogen as ammonia from casein		Nitrogen as nitrates from ammonium sulfate			
	pounds	*pounds*	*mgm.*	*relative*	*mgm.*	*relative*	*mgm.*	*relative*
XII	3,200	0	49.6	100	0.4	100	4.4	100
		2,000	70.5	142	1.4	350		
		5,000	93.0	187	2.0	500		
		10,000	89.5	180	3.2	800	5.6	127
XIII	3,800	0	46.0	100	1.1	100	5.4	100
		2,000	62.1	135	3.0	273		
		5,000	76.3	166	4.5	410		
		10,000	81.3	177	3.8	345	7.1	131
XIV	4,600	0	30.0	100	0.3	100	2.9	100
		2,000	45.5	152	0.7	233		
		5,000	72.4	241	1.1	367		
		10,000	86.3	288	0.7	233	4.4	152

been given in table 1 previously referred to. Analyses of the soil on the various plots were made and are recorded in table 13.

The studies in nitrification, ammonification, and nitrogen fixation were conducted in the same manner as previously mentioned in the discussion of the 12 soils of the Dekalb series with varying calcium-carbonate requirements. It will be remembered that the soil of the fertility plots is also Dekalb soil. The records of these experiments are shown in table 14.

TABLE 13

Analyses of soils of table 14

PLOT	TREATMENT	POUNDS PER 2,000,000 POUNDS OF SOIL			
		Nitrogen	Phosphorus	Carbon	Calcium-carbonate requirement
		pounds	*pounds*	*pounds*	*pounds*
19	N, P, K, CaO	2130	765	24,500	0
20	M, CaO	2700	1045	32,500	0
21	Check	1830	590	21,200	2800
22	CaO	1750	510	19,400	0
25	M	3240	1220	36,800	2800
26	N, P, K	2665	900	30,400	3200
28	P, K	2280	850	26,000	3600
29	N, K	2290	640	27,000	3400
31	N, P	2395	880	28,000	3200
32	K	2310	740	29,200	3600
34	P	2300	885	28,200	3400
35	N	2100	620	28,800	3400

N indicates nitrate of soda; P, acid phosphate; K, sulfate of potash; M, manure.

TABLE 14

The effect of calcium carbonate on the activities of soil bacteria in Dekalb soils which have received varying fertilizer treatments

TREATMENT	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS OF SOIL	CALCIUM CARBONATE APPLIED PER 2,000,000 POUNDS OF SOIL	NITROGEN PER 100 GM. OF SOIL				NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION	
			Nitrogen as ammonia from casein		Nitrogen as nitrates from ammonium sulfate			
	pounds	*pounds*	*mgm.*	*relative*	*mgm.*	*relative*	*mgm.*	*relative*
N, P, K, CaO.....	0	0	78.3	100	15.3	100	5.6	100
		2,000	80.1	102	18.5	121		
		5,000	78.3	100	19.5	127		
		10,000	77.4	99	20.3	133	7.5	134
N, CaO.....	0	0	87.8	100	17.5	100	6.2	100
		2,000	89.5	102	21.5	123		
		5,000	87.0	99	22.5	129		
		10,000	84.3	96	22.0	126	6.4	103
Check.....	2,800	0	60.6	100	1.2	100	3.7	100
		2,000	65.8	108	5.4	450		
		5,000	78.2	129	11.8	983		
		10,000	79.7	131	15.5	1275	4.2	113
CaO.....	0	0	71.8	100	8.5	100	2.6	100
		2,000	75.2	105	15.8	187		
		5,000	82.9	115	18.3	215		
		10,000	82.7	115	22.0	259	4.8	185
M.....	2,800	0	71.7	100	6.7	100	5.1	100
		2,000	75.3	105	12.5	186		
		5,000	90.5	126	16.3	243		
		10,000	90.0	125	21.5	321	7.2	141
N, P, K.....	3,200	0	70.1	100	2.9	100	4.2	100
		2,000	80.3	114	7.0	241		
		5,000	85.5	122	9.3	321		
		10,000	86.6	126	13.8	475	6.1	145
P, K.....	3,600	0	58.4	100	1.4	100	4.3	100
		2,000	66.2	109	4.3	301		
		5,000	76.3	131	8.5	601		
		10,000	82.4	141	13.0	928	5.5	128
N, K.....	3,400	0	56.6	100	1.5	100	5.1	100
		2,000	65.9	116	4.8	320		
		5,000	75.5	133	7.0	467		
		10,000	81.8	144	10.0	667	6.8	133
N, P.....	3,200	0	60.9	100	1.8	100	4.4	100
		2,000	68.7	113	5.8	322		
		5,000	82.9	136	9.7	504		
		10,000	85.2	140	12.8	701	5.5	125

TABLE 14—(Continued)

TREATMENT	CALCIUM-CARBONATE REQUIREMENT PER 2,000,000 POUNDS OF SOIL	CALCIUM CARBONATE APPLIED PER 2,000,000 POUNDS OF SOIL	NITROGEN PER 100 GM. OF SOIL				NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION	
			Nitrogen as ammonium from casein		Nitrogen as nitrates from ammonium sulfate			
	pounds	*pounds*	*mgm.*	*relative*	*mgm.*	*relative*	*mgm.*	*relative*
K	3,600	0	47.9	100	1.1	100	7.4	100
		2,000	57.4	120	2.5	237		
		5,000	75.7	158	5.0	454		
		10,000	84.0	174	6.6	600	7.6	103
P	3,400	0	49.5	100	1.1	100	5.3	100
		2,000	65.0	131	3.4	301		
		5,000	79.4	160	7.0	636		
		10,000	84.3	170	8.5	772	7.6	143
N	3,400	0	50.2	100	1.1	100	3.8	100
		2,000	64.9	129	2.9	272		
		5,000	78.6	156	5.5	500		
		10,000	83.1	165	7.3	663	6.9	182

Nitrification of ammonium sulfate was not very active in these soils except on the plots where lime had been applied in the field. Even the soil of plots 26, 28 and 31, which had been producing very satisfactory crops as indicated in table 1, did not contain vigorous nitrifying organisms. The rate of nitrification was materially increased by applications of calcium carbonate. The nitrifying organisms were much more active in the soil from the manure plots than in the soil from any of the other plots except where lime had been applied. There was a general tendency for the rate of ammonification of casein to decrease with an increase in the lime requirement of the soils. There were some marked exceptions to this tendency, notably plots 25 and 26. A study of the analyses of these plots shows a high total content of nitrogen and organic matter. No lime has ever been applied to plots 25 and 26. This increased nitrogen in the form of protein represents an increased amount of material available for the action of ammonifying organisms. If a large amount of nitrogen has been stored up in the soil, the amount of ammonia produced without any applications of calcium carbonate would be sufficient to produce satisfactory yields of those crops which are able to utilize ammonia, on a soil having lime requirements no higher than those of plots 25 and 26. The tendency for small applications of calcium carbonate to be relatively much more effective than larger applications was again shown in these experiments. It was evident that ammonification proceeds fairly satisfactorily without the application of calcium carbonate, especially, as suggested in the preceding discussion, if the content of organic nitrogen is high. Large applications of calcium carbonate had a tendency to reduce the rate of ammonification.

Nitrogen fixation took place very readily in Ashby's solution when inoculated with soil from any of the plots. There did not seem to be any correlation between the calcium-carbonate requirement and nitrogen fixation. The addition of calcium carbonate to Ashby's solution caused an increase in nitrogen fixation in every case, but this increase was no more marked in soil having a high lime requirement than in soil having a low lime requirement.

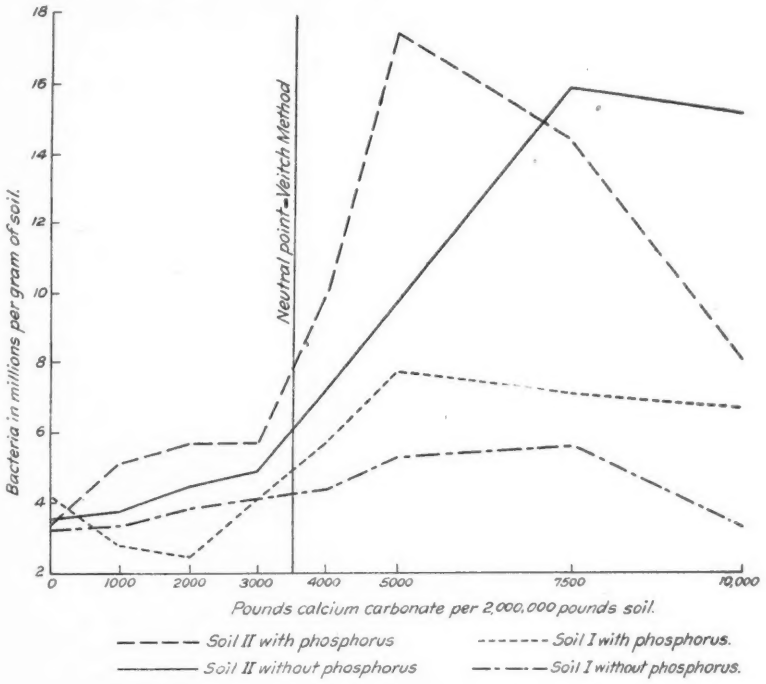

FIG. 1. THE EFFECT OF CALCIUM CARBONATE ON THE NUMBER OF BACTERIA IN SOILS I AND II

SUMMARY AND CONCLUSIONS

This investigation was undertaken as a preliminary step in the study of the possibilities of a system of acid agriculture on soils somewhat distantly removed from a source of lime. A study was made of the relation between the activities of the soil bacteria concerned in nitrogen accumulation and nitrogen transformations and the lime requirement of certain soils. The lime requirement of these soils varied from none to 4600 pounds of calcium carbonate per 2,000,000 pounds of soil. To different portions of these soils calcium carbonate was added in amounts ranging from 0.01 per cent to 2 per cent of the weight

of the soil. The data accumulated show that the various groups of soil organisms vary in their response to applications of calcium carbonate.

Ammonification proceeded fairly satisfactorily in most of the soils without the application of lime. The use of moderate amounts of calcium carbonate increased the rate of ammonification in most cases. Small applications were much more effective, relatively, than large applications.

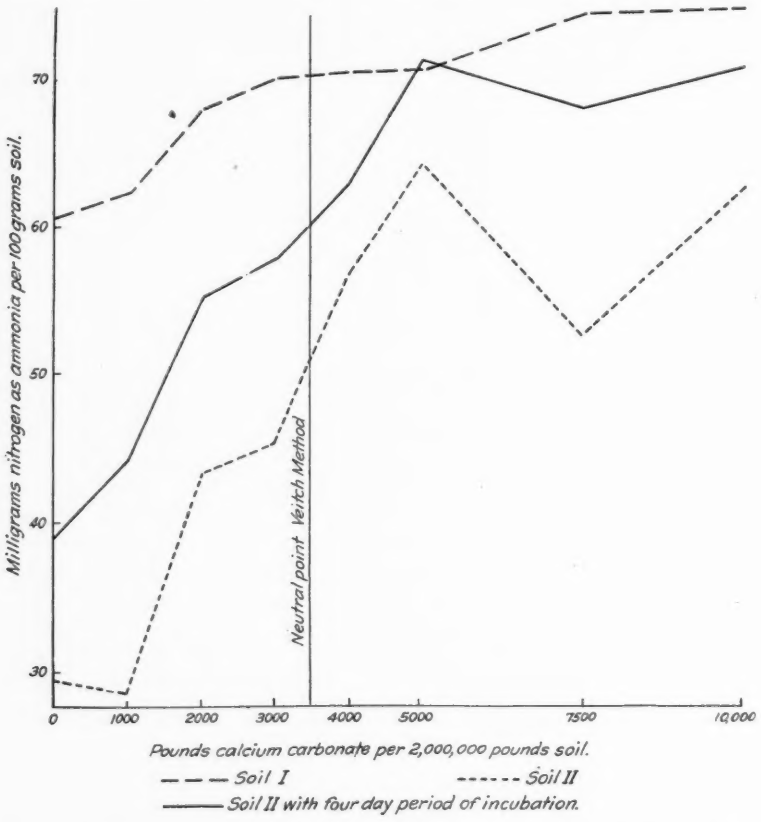

FIG. 2. THE EFFECT OF CALCIUM CARBONATE ON THE RATE OF AMMONIFICATION IN SOILS I AND II WITH PHOSPHORUS

The rate of nitrification was almost directly correlated with the amount of calcium carbonate supplied. Excessive applications were not injurious to the nitrifying organisms. Soils having high lime requirements showed practically no nitrification until calcium carbonate had been mixed with them.

Nitrogen fixation by non-symbiotic soil organisms was considerably in-

creased by the addition of calcium carbonate. The application of monocalcium phosphate also was necessary for maximum nitrogen fixation. All of the soils studied accumulated considerable amounts of nitrogen when incubated in Ashby's solution without the addition of calcium carbonate, although its use increased the rate of nitrogen fixation.

A lime requirement of 3000 pounds was not sufficient to prevent a good growth of soybeans on soil well fertilized with acid phosphate or manure. Nitrogen fixation accompanying the growth of soybeans took place readily

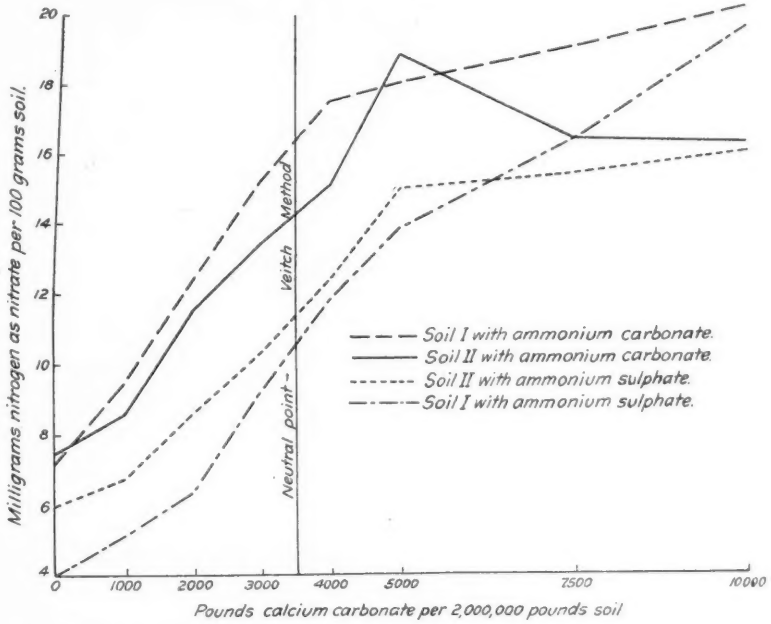

FIG. 3. THE EFFECT OF CALCIUM CARBONATE ON THE RATE OF NITRIFICATION IN SOILS I AND II WITH PHOSPHORUS

in acid soils. This fixation was increased by small applications but decreased by large applications of calcium carbonate.

From these facts the following conclusions seem justified:

1. Plants which are able to utilize ammonia nitrogen need not suffer from nitrogen hunger when grown on soils having lime requirements no higher than those studied in these investigations.

2. Plants which depend on nitrates as their source of nitrogen may suffer from the lack of available nitrogen in soils having high lime requirements, unless these requirements have been at least partially satisfied.

3. The supply of nitrogen in acid soils may be maintained by growing acid-resistant legumes, of which the soybean is one. Undoubtedly, the use of acid phosphate aids materially in the nitrogen-fixation processes in acid soils.

4. Small applications of calcium carbonate are, as a rule, relatively more effective than large applications as a means of increasing the bacterial activities in acid soils.

Acknowledgment is due Dr. E. B. Fred of the University of Wisconsin for many helpful suggestions and criticisms offered during the progress of this investigation.

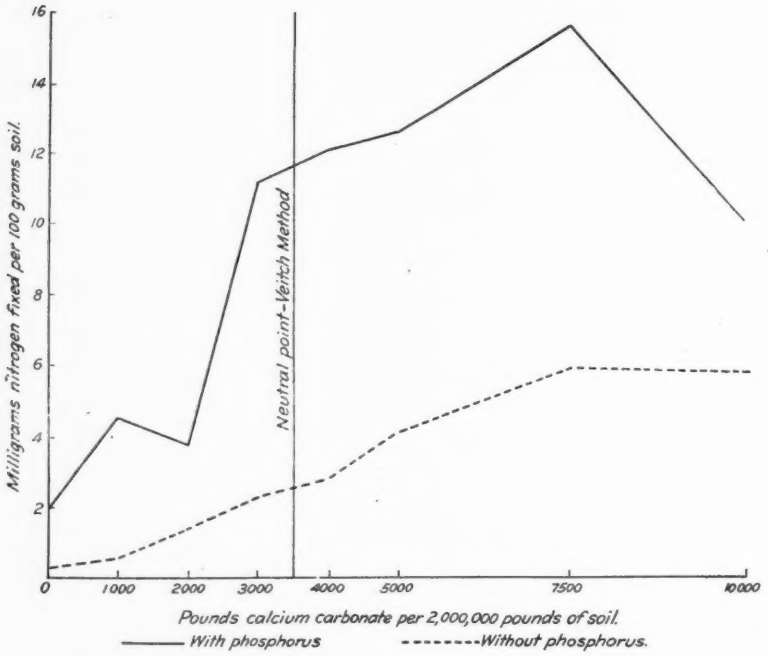

FIG. 4. THE EFFECT OF CALCIUM CARBONATE ON NON-SYMBIOTIC NITROGEN FIXATION IN SOIL II

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THE MOISTURE EQUIVALENT DETERMINATIONS OF SALT-TREATED SOILS AND THEIR RELATION TO CHANGES IN THE INTERIOR SURFACES

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The physical properties of soils in the field or laboratory may be appreciably modified by the addition of certain acids, bases, and salts. It is also true that a still more pronounced change in the soil properties will result if some of these added substances are washed either wholly or partly from the soil with water. To be consistent with the more recent theoretical reasoning, one must regard the changes in the degree of dispersion of the soil particles and the consequent alteration in the interfacial surfaces as important effects of such salt treatments. The more commonly noted effects as the changes in permeability, capillary activity, response to cultivation, and other similar soil factors are, we believe, to be properly regarded as incidental to and dependent upon the effects mentioned above.[1] Frequently, the exact relationship between these more obvious effects of salt treatments and the changes brought about in the interior soil surface are obscured by factors, the expressions for which have not been established. It is evident that those methods which most closely approach, either directly or indirectly, the measurement of the interior surface of soils are the most suitable for studies concerned with this phase of soil physics. Among the several methods which have been used in this laboratory for this purpose, the centrifugal determination of the moisture equivalent described by Briggs and McLane (1) seems to be one of the most promising.

Briggs and Shantz (2) have already pointed out the existence of important relationships between the moisture equivalent and the hygroscopic coefficient, the water-retaining power, and the mechanical analysis of soils. In addition they have correlated these coefficients with the wilting coefficient. Thus a single determination provides a comparatively simple, convenient, and fairly accurate means of connecting the physical properties of soils with their effects on biological processes. These reasons are sufficient to warrant a study of the salt effects on soils as indicated by the moisture-equivalent determinations. The first part of this paper presents the data secured by us

[1] Any effects which might be directly attributed to changes in the physical properties of the liquid phase, i.e., surface tension, vapor pressure, and density, are not considered in this discussion. We are dealing here with the changes in the solid phase, the soil particles.

in our studies. Aside from its usefulness in the directions already referred to, the moisture equivalent may be a means of obtaining a clearer conception of the comparative magnitudes of the interior surfaces of soils. At present this is purely a theoretical consideration and the few words devoted to it in the second part of this paper will suffice to explain it.

EXPERIMENTAL DATA

Duplicate 100-gm. portions of the Davis clay loam soil, which had passed the 2-mm. sieve were treated with 80 cc. of the salt solutions of the concentrations specified in table 1. From one of these portions the salt was washed with distilled water before centrifuging, while a sample from the other portion was subjected to centrifuging without washing. In numbers 19 to 21, inclusive, a sufficient quantity of the various salts was added to yield an amount of base equivalent to 3 gm. of sodium to 100 gm. of soil. Table 1 gives an outline of these treatments and the corresponding moisture equivalents.

The data given in table 1 show that the addition of NaCl and Na_2SO_4 to the Davis soil did not materially modify the moisture equivalent. In all probability the explanation of this lies in the fact that the smaller soil particles are flocculated to such a degree under normal conditions that the further addition of the flocculants NaCl and Na_2SO_4 proved to be without measureable effect on the moisture equivalent. On the other hand, it is likely that if a soil, the particles of which were in a state of diffusion, were substituted for the Davis soil, then more noticeable effects would result from the application of flocculating agents.

To formulate an explanation for the behavior of the Davis soil to which Na_2CO_3 and NaOH had been added is not as simple as in the case of the neutral salts. We would expect that these alkaline substances would increase, very markedly, the degree of dispersion of the soil particles. That this is not always the case is evident from some other experiments in which Na_2CO_3 (3) under certain circumstances was shown to lack the deflocculating power exhibited by NaOH toward suspensions of the Davis soil. This probably accounts for the similarity of the moisture equivalent of the soil treated with Na_2CO_3 and that of the control soil in the present experiments. As previously mentioned, NaOH of certain concentrations increases the degree of dispersion of the soil particles. A corresponding increase in the moisture equivalents of the soils treated with the proper concentrations of NaOH should logically follow. On the contrary, our experiments seem to indicate that the moisture equivalent is not changed by the addition of NaOH. We are not, at this time, in a position to offer a satisfactory explanation of this behavior. On the whole, it can be said that while the added salts are present in the soil, little or no change in the moisture equivalent was observed.

A very different effect is produced if these same salts are washed from the soil with water. The soils so treated seem to possess a new and peculiar set

TABLE 1

Effect of salts and washing treatments on the moisture equivalent of the Davis soil

NUMBER	SALT	CONCENTRATION	SALT ADDED PER 100 GM. OF SOIL	MOISTURE EQUIVALENTS			
				Salt present		Salt leached out	
				Duplicates	Average	Duplicates	Average
			grams				
1	00	00	00	19.06 19.46	19.26	19.34 18.97	19.15
3	NaCl	N	4.680	20.10 19.30	19.70	24.26 25.43	24.94
4	NaCl	N/10	0.468	19.20 20.00	19.60	22.08 22.21	22.14
5	NaCl	N/50	0.093	19.00 19.90	19.45	22.29 22.41	22.35
6	NaCl	N/100	0.046	20.10 19.30	19.70	21.53 21.39	21.46
7	Na_2SO_4	N	5.680	19.00 19.20	19.10	32.19 31.91	32.05
8	Na_2SO_4	N/10	0.568	19.80 19.70	19.75	22.97 22.81	22.89
9	Na_2SO_4	N/50	0.113	20.00 19.70	19.85	21.45 21.61	21.53
10	Na_2SO_4	N/100	0.056	20.20 20.10	20.15	20.73 20.42	20.57
11	Na_2CO_3	N	4.240	19.62 19.64	19.63	29.45 28.35	28.90
12	Na_2CO_3	N/10	0.424	20.01 20.03	20.02	20.03 20.00	20.01
13	Na_2CO_3	N/50	0.085	18.40 18.77	18.53	19.05 19.05	19.05
14	Na_2CO_3	N/100	0.042	18.86 18.74	18.80	18.69 18.70	18.69
15	NaOH	N	3.200	19.92 20.11	20.01	29.70 30.32	30.01
16	NaOH	N/10	0.320	20.52 20.07	20.29	22.24 24.13	24.18

TABLE 1 (Cont.)

NUMBER	SALT	CONCENTRATION	SALT ADDED PER 100 GM. OF SOIL	MOISTURE EQUIVALENTS			
				Salt present		Salt leached out	
				Duplicates	Average	Duplicates	Average
			grams				
				19.24		19.44	
17	NaOH	N/50	0.064	19.37	19.30	19.24	19.34
				18.58		19.43	
18	NaOH	N/100	0.032	18.73	18.65	19.44	19.43
						30.96	
19	$NaNO_3$	Salt added equivalent to 3 gm. of base per 100 gm.	11.078			30.32	30.64
						32.86	
20	$NaC_2H_3O_2$	of soil	15.620			29.60	31.23
						19.88	
21	$CaCl_2$		8.300			19.22	19.55

of physical properties. As shown in table 1 the moisture equivalent of the Davis soil is markedly increased by such treatments. The extent to which the moisture equivalent is changed depends upon the salt used. Thus the washing out of all the sodium salts was accompanied by a considerable increase in the moisture equivalent, while the washing out of $CaCl_2$ did not perceptibly alter this factor. Since the leaching out of other salts as KCl, K_2SO_4, KNO_3 and $(NH_4)_2\,SO_4$ produces an effect quite similar to that existing after the sodium salts have been leached from the soil, it is highly probable that the washing out of the salts first mentioned would produce effects on the moisture equivalent commensurate with those found when sodium salts have been leached from the soil.

The amount of change in the moisture equivalent due to leaching seems to depend also upon the anion with which the sodium is associated. In the experiment reported Na_2SO_4 produced the greatest effect, followed in order by NaOH, Na_2CO_3 and NaCl. Of course, equal quantities of sodium are under comparison.

The absolute quantity of salt with which the soil has been treated is likewise an important factor in determining the extent to which the soil will be modified. The larger applications followed with washing of salts invariably produced the greater effects on the moisture equivalent. The two smaller applications of Na_2CO_3 and NaOH were without a measurable effect. The application of some of the results of the investigations of Briggs and Shantz to our own problem of handling the salt-treated, water-washed soils has enabled us to see more clearly the reasons for the difficulties involved in

attempting to grow plants in such diffused soils, and have offered us valuable suggestions as to the proper procedure for alleviating these conditions.

THEORETICAL DISCUSSION

The optimum physical conditions for plant growth in such heterogeneous mediums as the soil mass must obviously depend in some measure upon the interfaces between the various phases and the factors affecting them. As mentioned in the first part of this paper, one of these factors is the effect of salts on the degree of dispersion of the soil particles. Furthermore, in many cases the leaching out of salts is instrumental in bringing about a greater change in the degree of dispersion of the soil particles than that produced by the mere addition of the salts, and hence it would seem that the salt and water treatments would also be accompanied by greater changes in the interfacial surfaces. These statements serve to introduce some of the intricacies of the problems involved in the measurement of surfaces within the soil mass, especially those affected by salts.

We now propose to consider the moisture equivalent determinations as functions of the interior surfaces of soils. Our assumption presupposes that the moisture equivalent varies directly as the surface of the solid soil particles. Such a relationship would be the logical deduction from the following considerations.

From the moisture equivalent it is possible to obtain the volume of water which a given quantity of soil will retain against a given force. According to Briggs and McLane (1) the water so held is in capillary equilibrium. That is to say, the water films in any two soils, which have been centrifuged, are of equal thickness and the liquid-air surfaces in the capillary spaces are of the same curvature.

By momentarily neglecting the possibility that the only inequality that any two soils may show with respect to their residual water content after centrifuging, lies in the number of points of contact between particles, or the number of capillary spaces, we are enabled to consider the water usually designated by the term "moisture equivalent" to be spread over the surfaces of the two soils to an equal depth, and hence the surfaces would vary directly as the volume of water retained.

Thus let X be the thickness of the water film and A and B the volumes of water retained by two soils. Then the surfaces S_a and S_b are as follows: $S_a = A/X$ and $S_b = B/X$, and S_a is to S_b as A is to B. If a finite value could be assigned to X, then the figures for S_a and S_b would approach absolute values.

The most serious objection to such a line of reasoning lies in the factor which we purposely neglected for the moment, namely, the number of capillary spaces in which water may collect to a depth greater than that in the surface films. Obviously, in such cases the thickness of the water layers

would not be uniform throughout, which possibly invalidates any rigorous mathematical interpretation of the data obtained by the use of the centrifuge. On the other hand, the differences in thickness may not be so great as to militate seriously against the correctness of the general proposition that the moisture equivalent is a more or less accurate function of the interior surface.

But there are certain compensating factors which would tend to equalize the thickness of the water layers, even though the number of points of contact between particles should be increased. This can best be shown by the following sketch:

In *A* two spherical particles are shown suspended in a liquid some of which has become closely associated with the surfaces of the particles. If the excess liquid is gradually removed the particles come closer together and the liquid films around them assume a form as in *b*. In the immediate region between the two spheres the thickness of the water films is somewhat decreased, while further out from the spheres the film thickness is increased. *C* represents the final appearance of such a system when the excess water is removed by some force as that developed by centrifuging. In this case the

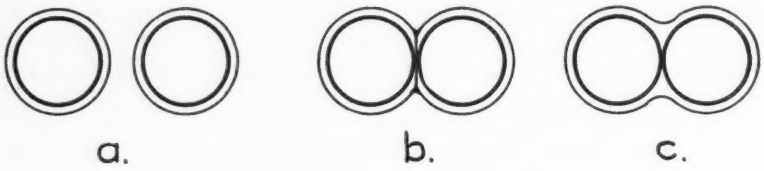

FIG. 1

particles are in contact with each other, necessitating a marked decrease in the thickness of the film adjacent to the point of contact. A further examination of this system shows, however, that this decrease in thickness may be largely compensated for by a corresponding increase in the thickness of the water layers in the capillary spaces. In other words, the total volume of water retained and the actual surface of the particles are not materially changed by increasing or decreasing the number of points of contact between the particles. Hence, the average thickness of the water film surrounding the soil particles would likewise be undisturbed. Evidently, the extent of the surface over which the residual water is to be spread is of more importance in determining the volume of water retained than the number of capillary spaces. The latter statement refers to soils having particles of the same size but differing in the arrangement of these particles. Of course, any change in the actual size of the particles or even in their effective size necessarily means change in the interior surface of the soil, and hence a change in the moisture equivalent. It is for the reasons outlined above that the moisture equivalent is considered as an index of the interior surface of soils.

A second objection to our proposal lies in the fact that the conditions of an ideal system composed of spheres of the same size and pure water are not fulfilled by the soil system, for the soil particles are not spherical, nor are they of uniform size; hence the laws concerning the first type of system may not hold with the same exactitude for the soil-water-air system. Although the soil particles may be variously shaped, the indentures and other irregularities on their surfaces would act just as points of contact accompanied with interstitial spaces, so that, in the main, the surface of the particle would be the primary factor in the retention of water against any force.

SUMMARY

With these points in view we may conclude that the salt and water treatments have increased the interior surface of the soils from 2 to 40 per cent, the magnitude of the increase depending upon factors which have already been mentioned. The salts alone have not measurably affected the interior surface.

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RELATION OF THE MECHANICAL ANALYSIS TO THE MOISTURE EQUIVALENT OF SOILS

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The soil physicist has been very much interested during the past decade in trying to find some constant that will be a measure of the physical properties of soils so that when comparing two or more soils, this so-called constant will express numerically the difference that may exist between such soils.

Comparatively little work has been done by use of the moisture-equivalent determination and since some writers have claimed that there is a direct relation between the mechanical analysis of a soil, for instance, and its moisture equivalent, it was thought desirable to present a preliminary report at this time showing some results that have been obtained in the laboratory of the division of soil technology of the University of California, and to endeavor to offer explanations of the results.

The moisture equivalent was determined by use of the centrifuge designed by Briggs, et al, (2, 3) and was operated at a speed of 2400 revolutions per minute for a period of 30 minutes. This machine exerts a force 1000 times that of gravity on the saturated soil contained in the centrifuge cups.

The mechanical analysis was made by the Bureau of Soils method (4, 6) and special care was exercised to insure that the separation into the seven groups was made as accurately as possible. The analysis was made on 12 different soil types in duplicate, ranging in texture from coarse sand to clay and in origin from residual to recent alluvial. All of the particles belonging to the same group were combined so that a composite sample for each of these seven groups would be obtained. The moisture equivalent for these composite groups of soil particles was then determined in duplicate and not calculated indirectly, as most previous investigators have done.

The results obtained from the moisture-equivalent determinations of these seven grades of soil particles are given in table 1.

Specific-gravity determinations were also made on these seven grades. The results obtained are given in table 2 and show a range from 2.64 to 2.69 and seem to indicate that the separation by the mechanical analysis gave seven grades of soil material that differed mainly in the size of the particles constituting any one group, and not in any marked variation in mineral content.

By noting the results obtained for the different-sized particles making up a soil, as given in table 1, it can readily be seen that textural grade has a definite influence on the moisture equivalent of the soils and that each grade has a definite capacity for holding moisture.

There are soils in the western part of the United States which contain a large proportion of particles ranging from 0.05 mm. to 2 mm. in size, with a relatively small amount of silt and clay. They are, however, capable of holding sufficient moisture for the successful maturity of crops such as grapes, in regions where the annual rainfall averages below 15 inches and no irrigation is resorted to.

TABLE 1

Results obtained in moisture-equivalent determinations

TEXTURAL GRADE	SIZE OF PARTICLES	MOISTURE EQUIVALENT
	mm.	*per cent*
Fine gravel	2.0 –1.0	1.18
Coarse sand	1.0 –05.	1.44
Medium sand	0.50 –0.25	1.85
Fine sand	0.25 –0.10	2.34
Very fine sand	0.10 –0.05	4.62
Silt	0.05 –0.005	24.99
Clay	0.005–0.0001	61.03

TABLE 2

Results obtained in specific gravity determinations

TEXTURAL GRADE	SPECIFIC GRAVITY
Fine gravel	2.67
Coarse sand	2.64
Medium sand	2.64
Fine sand	2.69
Very fine sand	2.66
Silt	2.65
Clay	2.66

The above results indicate that if it is possible to use the mechanical analysis as an indirect method for the calculation of the moisture equivalent, the investigator must give to each textural grade a definite and distinct value and not disregard the sands, or group three or four grades into one.

Three synthetic soils were then made up from these grades of soil particles, following the average mechanical analysis for these three soils as given by the United States Bureau of Soils (8), and the moisture equivalent was determined for these synthetic soils by the usual method. Before determining the moisture equivalent, however, the soils were allowed to stand in a moistened condition for ten days to allow a thorough saturation and an adjustment in the structure of the soil mass. The mechanical analysis and the moisture equivalent of these synthetic soils are shown in table 3.

Various formulas have been proposed for the calculation of the moisture equivalent from the mechanical analysis, of which the following two have received the most prominence:

Briggs (5) proposed the values

0.02 sands + 0.22 silt + 1.05 clay = moisture equivalent,

and Alway and Russell (1)

0.14 sands + 0.27 silt + 0.53 clay = moisture equivalent.

The results obtained up to this time in our laboratory agree more closely with the latter formula than with the former. By noting the results given in table 1 it will be found that the sands total 0.11, the silt 0.25 and the clay 0.61.

TABLE 3

Mechanical analysis and moisture equivalent of three synthetic soils

SOIL TYPE	FINE GRAVEL	COARSE SAND	MEDIUM SAND	FINE SAND	VERY FINE SAND	SILT	CLAY	MOISTURE EQUIVALENT
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Loam soil	2	5	5	15	17	40	16	21.02
Sandy loam	4	13	12	25	13	21	12	14.50
Fine sandy soil	1	4	10	57	17	7	4	6.65

TABLE 4

Determined moisture equivalent compared with calculated moisture equivalent

SOIL TYPE	MOISTURE EQUIVALENT		
	Determined	Calculated	Departure
Loam soil	21.02	20.86	−0.16
Sandy loam soil	14.50	14.01	−0.49
Fine sandy soil	6.65	6.32	−0.33

Taking the values for the moisture equivalent as determined for each separate and then calculating the moisture equivalent by using these values in conjunction with the mechanical analysis of the synthetic soils and comparing the result with the actual moisture equivalent of the synthetic soil, the value of a formula can be tested. The averages used for the various textural grades on the basis of the results given in table 1 were: fine gravel 0.010, coarse sand 0.015, medium sand 0.020, fine sand 0.020, very fine sand 0.045, silt 0.250, and clay 0.600.

The results of the determined moisture equivalent compared with the calculated moisture equivalent for the three synthetic soils are given in table 4.

If the sands are totaled the departure of the calculated moisture equivalent from the determined moisture equivalent is much greater than if definite values are given to each of the sand groups, as was done in the table.

The results given in table 4 show that the calculated moisture equivalent

is practically the same as the determined when separate values are given to the seven individual grades of texture, and not when determined by totaling the five grades of sand or disregarding the sands and just considering the silt and clay content of a soil.

A mechanical analysis was then made on 18 samples of soil, the moisture equivalent determined by use of the centrifuge as well as calculated, and the departure noted. The calculated moisture equivalents were in nine cases below the determined and in the others above. The departure of the calculated moisture equivalent from the determined ranged from -0.90 to $+2.94$.

On a similar set of samples (ten in number) the mechanical analysis and the moisture equivalents were determined by some advanced students in our laboratory. The departure of the calculated moisture equivalent from the determined was greater than that found in the previous set and ranged from -5.44 to $+5.31$.

For the third set of samples the mechanical analysis for 30 samples of soil were obtained from the report of the Bureau of Soils, The Soil Survey of the Ukiah Area, California. Duplicate samples of each of these soils were in our laboratory and the moisture equivalent was determined on them. The calculated moisture equivalent departed from the determined from -0.57 to $+7.28$.

There were several samples of special interest in that the mechanical analysis was practically the same, yet the determined moisture equivalent varied considerably. The results for some of these peculiar samples are given in table 5.

The mechanical analyses of the first two samples given in table 5 are almost identical, but the determined moisture equivalent of one is 30.80 and for the other 35.82. A similar condition exists for the other two samples noted in the same table.

Taking the average mechanical analysis of several representative soil classes as given by the Bureau of Soils (8) and calculating their moisture equivalent by using the values for the various textural grades as given in table 1, the results shown in table 6 were obtained.

Table 6 shows that from the average of a large number of mechanical analyses there is a direct relation between texture of the soil and its moisture equivalent.

Alway and Russell state (1) "If the mechanical analysis is to be used for the indirect determination of the moisture equivalent, it will be necessary at least in the case of some widely-differing soil types to employ several different formulas."

From the results given in this and previous publications it becomes evident that one formula will not hold in all cases, if that formula is calculated by means of least squares as was done by Briggs and McLane (2, 3) or by direct determination of the moisture equivalent for the various separates, as was tried in this laboratory.

One factor that has been overlooked by most investigators has been the influence of the shape of the soil particles on the moisture retentiveness of soils or on their moisture equivalent. Free states (7), "A very micaceous soil, for instance, has very different physical properties from one of the same mechanical analysis but composed of particles which are mainly spheres instead of disks." The results reported in this paper for the three synthetic soils show that the calculated moisture equivalent agrees very closely with the determined moisture equivalent, keeping in mind that the same sepa-

TABLE 5

Mechanical analysis and moisture equivalent of four special samples of soils

DESCRIPTION OF SAMPLE	MECHANICAL ANALYSIS							MOISTURE EQUIVALENT DETERMINED
	Fine gravel	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt	Clay	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Residual surface soil (Climax clay adobe)	2	2	1	6	9	33	47	30.80
Residual subsoil (Climax clay adobe)	1	3	1	6	9	34	46	35.82
Recent alluvial surface soil (Yolo silty clay loam)	1	3	2	8	14	47	25	25.97
Recent alluvial subsoil (Yolo silty clay loam)	2	3	2	11	14	45	23	21.33

TABLE 6

Calculated moisture equivalents of seven soils

SOIL TYPE	MOISTURE EQUIVALENT CALCULATED
Coarse sand	6.38
Sandy loam	14.00
Fine sandy loam	15.04
Loam	20.85
Silt loam	25.91
Clay loam	26.11
Clay	34.82

rates were used for the derivation of the formula as were used in making the synthetic soils. When one, however, applies the results obtained to field soils varying widely in age and origin, the results are far from being in agreement.

It was thought at first by the writer that it would be possible to have one formula to be used for residual soils, another for "wind-laid," another for recent alluvial, etc.; which might take care of the shape of the soil particles. When one, however, sees how the surface soil of residual origin as given in table 5 has the same mechanical analysis as the subsoil of the same origin

and yet a considerably lower moisture equivalent, while on the other hand, a recent alluvial surface soil has the same mechanical analysis as its subsoil yet a considerably higher moisture equivalent, it is evident that any suggested formulas for calculating a constant such as the moisture equivalent from the mechanical analysis of soils are far from accurate.

The mechanical analysis just considers the amounts of the various-sized particles in a given soil, while the moisture equivalent as determined by means of the centrifuge gives a soil constant which is influenced not only by the size and amount of the different particles present in a soil, but also by the shape of the soil particles, amount of organic matter present, amount of soil colloids present, chemical composition of the soil, etc.

From the data given it is felt that while the moisture equivalent calculated from the mechanical analysis according to the formulas suggested gives approximate results, nevertheless they are far from accurate for scientific work, and it will be necessary to make an actual moisture-equivalent determination for satisfactory results.

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THE VOLUMETRIC DETERMINATION OF SULFATES IN WATER EXTRACTS OF SOILS

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The investigation of the water-soluble plant-food elements in soils offers one of the most fruitful methods of attacking problems in soil fertility. Of these elements, considerable interest has been evinced of late in the sulfur requirements of crops and the use of sulfur fertilizers. A number of recent articles on this subject suggest that possibly the sulfur nutrition of plants has not received sufficient attention. During recent investigations in this laboratory as reported by Stewart (4), it became desirable to ascertain the exact amounts of sulfates present in water extracts of cropped and uncropped soils at all seasons of the year.

For a correct determination of the elements extracted from soils by water, the usual gravimetric or volumetric methods do not suffice, owing to the minute quantities present. The first compilation of special methods used in the analysis of water extracts of soils was presented by the Bureau of Soils (3) in 1906. Of these methods, the turbidity method for sulfates as described by the authors, Schreiner and Failyer, was first used. The determination of sulfates by this method consists in comparing the turbidity produced by the addition of barium chloride to the water extract with that produced in a standard sulfate solution treated similarly. After a thorough trial this method was discarded by us, inasmuch as concordant results were not obtained. When from 10 to 20 parts per million of sulfate were present, fairly accurate results were obtained but with less than 10 parts per million the results were generally 30 to 40 per cent in error.

The colorimetric method for sulfate as described by Winkler (5) and quoted by the Bureau of Soils (3) also was investigated. This method consists in precipitating the sulfate as barium sulfate by means of barium chromate in acid solution and upon neutralizing, an amount of chromate equivalent to the precipitated sulfate remains in solution. This is estimated by comparison with a standard chromate solution. Owing to the fact that barium chromate has a solubility of one part in 46,400 parts of water, it was found necessary to correct the final result by subtracting 8.2 parts per million from the number of parts per million of sulfate found. Since nearly all the soils used in this investigation, on being extracted with from 1 to 5 times their weight of water,

yielded solutions containing less than 8 parts per million of SO_4, the use of this method for small amounts of sulfate was undesirable.

Our attention was called to a volumetric method for the estimation of small amounts of sulfate in urine, as described by Raiziss and Dubin (2). This method depends upon the separation of the sulfate as insoluble benzidine sulfate followed by titration with N/10 $KMnO_4$. A recent publication by Drummond (1) advocates titration with N/50 KOH. The permanganate titration, however, seems preferable for very small amounts of precipitate, owing to the fact that a relatively larger amount of permanganate is required for titration (approximately 17 times more N/50 $KMnO_4$) than N/50 KOH for the same amount of benzidine sulfate). The method of Raiziss and Dubin (2) has been modified by us for the determination of small amounts of sulfates in water extracts of soils. In the investigations referred to above (4), 200 cc. of a 1 to 5 extract, representing 40 gm. soil, has been found in all the 14 soils examined, to contain sufficient sulfate for a convenient determination, using N/20 $KMnO_4$. The amount of extract employed should be chosen so as to contain not less than 0.1 mgm. nor more than 5.0 mgm. of SO_4. The method follows.

Evaporate a suitable aliquot to dryness in a 200 cc. casserole on the steam bath. Ignite at a low heat to destroy organic matter. Take up with about 5 cc. of dilute HCl (1:9), digesting on a steam bath for 5 to 10 minutes. Filter through a 5.5 cm. filter into a 500-cc. wide-mouthed Erlenmeyer flask, washing with several successive small portions of hot water until the volume amounts to 15 to 20 cc. Cool. Add one drop of phenolphthalein indicator and make just alkaline with NaOH (10 per cent). Acidify with one drop of HCl (1:4). Add 10 cc. of benzidine hydrochloride (8 gm. per liter of water) and allow to stand 15 minutes with occasional shaking. Filter with gentle suction on a small asbestos pad supported by a porcelain plate in a glass filter tube. Wash with three 5-cc. portions of cold water. Return the asbestos and precipitate to the flask by means of a jet of water and make the volume to about 200 cc. Add 1 cc. of 10 per cent NaOH and boil for 5 minutes. Cool to room temperature, add 20 cc. of concentrated H_2SO_4 and titrate with N/20 $KMnO_4$ until a pink coloration persisting 20 seconds is obtained. During the titration, the strong yellow color which first appears gradually fades and the solution becomes practically colorless before the end point is reached.

Raiziss and Dubin (2) state that under the above conditions 1 cc. of N/10 $KMnO_4$ equals 0.099 mgm. of S. By calculation, 1 cc. of N/20 $KMnO_4$ equals 0.15 mgm. of SO_4, which is taken as the factor. Next to a careful adjustment of the acidity, the most important precaution has been found to be that of washing. When less than 15 cc. of wash water were used, high results indicated that the excess reagent had not been entirely removed while washing much in excess of 20 cc. caused low results, due to the slight but appreciable solubility of benzidine sulfate.

In order to test the accuracy of the method, sulfate solutions of known con-

centration were analyzed. These solutions had been carefully prepared by dilution of stronger solutions checked by the usual barium chloride method. These results as well as 6 analyses of a typical 1 to 5 soil extract are given in table 1. The method is believed by us to be more dependable than the turbidity or colorimetric methods previously described, especially for relatively small amounts of sulfate.

TABLE 1

Determination of sulfate by volumetric method

	ANALYSES OF KNOWN SOLUTIONS						ANALYSES OF A SOIL EXTRACT	
	N/20 $KMnO_4$	SO_4	N/20 $KMnO_4$	SO_4	N/20 $KMnO_4$	SO_4	N/20 $KMnO_4$	SO_4
	cc.	*p.p.m.*	*cc.*	*p.p.m.*	*cc.*	*p.p.m.*	*cc.*	*p.p.m.*
Theoretical	1.0	0.75	10.0	7.50	20.0	15.00		
Found	0.8	0.60	9.5	7.12	19.6	14.70	4.9	3.67
Found	0.9	0.68	9.6	7.20	20.0	15.00	4.8	3.60
Found	1.1	0.83	9.6	7.20	19.9	14.92	4.8	3.60
Found	0.9	0.68	9.8	7.35	19.8	14.85	4.7	3.53
Found	1.1	0.83	9.7	7.27	19.9	14.92	4.8	3.60
Found	1.0	0.75	9.7	7.27	20.5	15.37	4.7	3.53
Average	0.97	0.73	9.65	7.23	19.95	14.96		
Maximum error	20 per cent		5.0 per cent		2.5 per cent			
Average error	3 per cent		3.5 per cent		0.25 per cent			

SUMMARY

The volumetric determination of small amounts of water-soluble sulfates in soils by titration of the precipitated benzidine sulfate with potassium permanganate is described.

The method is shown to have an average error of 3 per cent and is believed to be superior to colorimetric or nephlometric methods, especially for small amounts of sulfate.

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A STUDY OF THE ROOT-NEMATODE (HETERODERA RADICICOLA) AND ITS CONTROL

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INTRODUCTION

The root-nematode, eel-worm or root-gallworm, has long been known in the United States, particularly in soils of the South and occasionally in soils of the northern states. It is also widely distributed in greenhouses and the direct or indirect damage done by the nematode is very great. Up to date, an entirely satisfactory remedy has not been found, consequently this aspect of the problem offers a rich field for investigation. At the outset, then, it was necessary to consider all possible phases, and as a result certain points are revealed in this study which are of general interest, but these do not directly affect or concern the problem of practical control.

Since the discovery of the root-nematode in 1855 by Rev. M. J. Berkeley, it has been reported as occurring on 480 different species of plants, some of which are the tomato, celery, beet, lettuce, lima bean, radish, spinach, egg-plant, gardenia, carnation, sweet-pea, rose, violet, ginseng and other plants which are not so common.

Nematode infestation becomes noticeable when the plants show dwarfing and have a general unhealthy appearance. In some cases, however, infestation is not apparent until the roots are examined. An infested root is usually enlarged at the tip as a result of the irritation produced by the nematode. In this respect it differs from the round and lateral tubercles of bacteria formation, and furthermore, the swellings due to the root-nematode are elongated and pear-shaped.

The larva, or early stage in the animal's development, is a slender transparent eel-like organism which moves about by a whipping process. In a liquid containing no sediment this thrashing about does not produce a change of location while in a solution containing small particles the thrashing results in locomotion due to friction against the particles. The larva is about 400μ or approximately 0.5 mm. in length and nearly 15μ in width. It is colorless, but the body-wall and particles of food within the animal serve to differentiate it from the medium in which it is located. The larva, on hatching from the egg, searches for a growing root and upon finding it, enters at the growing tip and a gall is subsequently formed. Many larvæ may enter a single root but

the size of the gall and the number of larvæ within are not necessarily correlated.

While in the root, the larvæ undergo several molts and become true males and females. The mature male is similar to the larva in form and appearance. It is, however, much larger, being about 1400μ in length and 30μ in thickness (fig. 1). The female becomes pear-shaped and is from 400 to 1000μ in length, of a pearly white color. After being fertilized by the male the eggs begin to form.

Brown, oval eggs are forced out by the female. These eggs measure about 90μ in length and 40μ in thickness. Repeatedly, larvæ were observed coiled within the thick shell of the egg. (Fig. 1.)

Under average greenhouse temperatures, the eggs hatch in 5 days and the newly-hatched larvæ may immediately attack susceptible plants. The entire life-cycle in a greenhouse is completed in about 30 days.

The larvæ are capable of encysting and thereby capable of resisting adverse conditions, such as drought and certain chemicals. The eggs are likewise very resistant and this stage is able to withstand many strong chemicals.

Before 1898 (10) very little attention had been given to the study of nematode control; the limited amount of investigation before this period dealt mainly with the morphology and physiology (1). From the standpoint of control, steam sterilization and some chemicals have been tested (2, 3, 7, 8). Satisfactory control is claimed by steam sterilization of the soil but this method is in many cases impossible or at least impracticable. A large number of chemicals (8, 10) has been tried but so far as known none has given satisfactory results. In view of the above facts, it was thought necessary to consider all phases of this problem that time would permit.

EXPERIMENTS

Moisture

The first experiment tried was to determine the effect of moisture on root-nematode activity.

In the preliminary experiments with moisture it was seen that galls, taken from infested gardenia plants and placed in water over a source of mild heat, produced very active nematodes. For further study galls of approximately the same size (3.5 x 3 mm.) were thoroughly washed in distilled water and placed singly in stender dishes and each was treated, in a duplicate series, with different quantities of distilled water and maintained at a constant temperature for 72 hours and examined under the low power of a microscope at different intervals of time. All observations were from 1 hour to 72 hours. The results were the same at the end of 1 hour as at the end of 72 hours. The observations are given in table 1. In the table, Series A and B are duplicates and have the same treatment.

Another moisture experiment was made to confirm the preliminary one. This was run in triplicate series, each series having the same treatment. Results are given in table 2.

It can be seen from table 2 that the activity of the root-nematode is increased, the temperature remaining constant, with an increase in moisture

TABLE 1

Effect of moisture on root-nematode activity (at 21°C.)

WATER	SERIES A	SERIES B
cc.		
0.0	Inactive	Inactive
0.1	None out	None out
0.2	Very few out and slightly active	None out
0.3	Very few out and active	Few out and slightly active
0.4	Very few out and active	Few out and slightly active
0.5	Many out and active	Many out and very active
0.6	Many out and not active	Many out and few active
0.7	Immerged, few slightly active	Immerged but inactive
0.8	Immerged inactive	None seen
0.9	None seen	None seen
1.0	None seen	None seen
2.0	None seen	None seen
5.0	None seen	None seen
10.0	None seen	None seen

TABLE 2

Effect of moisture on root-nematode activity (at 19°C.)

WATER	SERIES A—LARVÆ	SERIES B—LARVÆ	SERIES C—LARVÆ
cc.			
0.0	Inactive	Inactive	Inactive
0.2	One out and active	One out and active	None seen
0.3	Slightly active	Slightly active	Slightly active
0.4	Some very active	Slightly active	Some very active
0.5	Many very active	Many active	Some very active
0.6	Many very active	Many active	Some very active
0.7	Many very active	Many active	Slightly active
0.8	Few active	Slightly active	None seen
0.9	None seen	Slightly active	None seen
1.0	Slightly active	Slightly active	Inactive

until an optimum moisture content is reached, and when continued above that point the activity is decreased.

In the above experiments, sterile water was used in order to eliminate the toxic substances, such as chlorine, copper salt, etc., found in tap water. This introduced another factor—that of plasmolysis. It was suggested that the concentration of sterile water was below the concentration of the liquid in the

body of the nematode and therefore plasmolysis might occur. This theory was tested by placing gardenia galls in a series of stender dishes containing sterile water and a similar series using water extracted from the soil—the natural solution in which the nematode normally lives. It was observed that the results from both solutions (0.2 to 1.0 cc.) compared very closely.

This proved that sterile water had no plasmolyizing effect used in rates up to 1 cc., and that the experiments were therefore sufficiently accurate.

Also, in connection with this experiment it was thought that because a definite number of larvæ were not used in each stender dish results would not be accurate. This possibility of error was dismissed on the ground that sufficient numbers (200 to 400) of larvæ were present in each dish to give safe averages. In every test the gall was crushed in order to reveal the dormant larvæ in observations recorded as "none seen." The fact that some larvæ emerged and others did not may be due to their location in the gall, that is, some of them were not near enough to the surface of the root to be affected by the moisture. The galls became submerged with 0.7 cc. of water.

If in the above tables on moisture, "inactivity" means death, then flooding the soil might offer a remedy for nematode infestation. Furthermore, it has previously been observed in the South that the root-nematode does not occur in bogs or water-soaked lands. Many observers have noticed that the nematode occurs mostly in sandy soils and not in clays. This may be due to the fact that sand does not retain water for any considerable length of time and thus allows sufficient aeration for the nematode. On the other hand, clay soils retain moisture and thus reduce the amount of air held. For the majority of cases, therefore, flooding was considered impracticable, and an unsatisfactory control, except perhaps under special conditions, and was dismissed from further consideration.

Temperature

The next test made was to determine the effect of temperature on nematode activity.

As in the experiment on moisture, gardenia galls of approximately the same size (3 mm. x 3.5 mm.) were immersed in 0.4 to 1.0 cc. of distilled water and triplicate series placed under three different temperatures. To keep the moisture constant the stender dishes were placed in tin trays containing water, and were covered with cheese-cloth which rested in the water and was kept moist by capillary attraction.

Series I was placed out-of-doors, sheltered from rain. The temperature averaged about 8.8°C., or 48°F.

Series II was placed in an electric incubator at a temperature from 36.6° to 38.8°C., or 98° to 102°F.

Series III was placed in a greenhouse at a temperature of 26.6°C., or 80°F.

The contents of all dishes were examined at the end of 24 hours under the

low power of a microscope. The results are given in table 3. Here again Series A, B and C have the same treatment.

In the above table "none out" and "none seen" indicate that no larvæ had emerged, but at the end of this experiment each gall was crushed and inactive larvæ were found.

The observations recorded in table 3 seem to warrant the conclusion that the optimum temperature for the nematode is between 65° and 85°F. Generally speaking, temperatures above 65°F. increase nematode activity, and temperatures below 65°F. and above 85°F. decrease nematode activity. The animal may be killed by freezing (32°F.), and by heating to 101°F. the larvæ and eggs are killed.

TABLE 3

Effect of temperature on root-nematode activity

	WATER	I (AT 8.8°C.)	II (AT 36.6° TO 38.8°C.)	III (AT 26.6°C.)
	cc.			
Series A ...	0.4	Out and active	Out and inactive	Out and inactive
	0.6	Out and active	Out and inactive	None out
	0.8	None out	Out and inactive	Out and slightly active
	1.0	Out and active	Out and slightly active	Out and slightly active
Series B ...	0.4	Out and active	Out and inactive	Out and very active
	0.6	Out and active	Out and inactive	Out and very active
	0.8	Out and active	Out and inactive	Out and very active
	1.0	Out and active	None out	Out and very active
Series C ...	0.4	Out and active	None out	Out and very active
	0.6	Out and active	Out and active	Out and very active
	0.8	Out and active	Out and inactive	Out and very active
	1.0	Out and active	None seen	Out and very active

The above results suggested that raising the temperature of the greenhouse soil to 101°F. would kill the larvæ and eggs. This however did not appear to be practicable for most greenhouses. High temperature may be applied with some degree of success at least, by exposing infested soil to the hot rays of the sun and turning it over to insure thorough sterilization.

Chemicals

Failing to discover a satisfactory remedy for nematode attack in the study of moisture and temperature conditions, attention was next directed to the study of chemicals. At the outset it was seen that a great many of the favorite chemicals used in soil sterilization could be omitted, for J. A. McClintock (8) has shown that the nematode could not be satisfactorily combated by any of the following: carbon disulfide, corrosive sublimate, calcium carbide, ammo-

nium sulfate, formalin, nicotine, benzine or kerosene. Furthermore, Stone and Smith, in Massachusetts (10), demonstrated that the following chemicals are ineffective: manganese sulfate, common salt, potassium nitrate, magnesium sulfate, calcium sulfate, kainit, sodium nitrate and lime.

The recent success with sodium cyanide (NaCN) obtained by Dr. T. J. Headlee of the New Jersey Agricultural Experiment Station, as a soil sterilizer in the control of wire-worms suggested that the same treatment might be used in combating the root-nematode.

Gardenia galls were placed in stender dishes as in experiments with moisture and temperature and treated with amounts of cyanide, ranging from 0.7071 gm. to 0.0707 gm. dissolved in 10 cc. of water, giving solutions from 0.7 to 7 per cent concentration. On standing 48 hours the larvæ had completely disappeared. This disappearance may have been due to disintegration. The eggs also were attacked and made colorless.

The above experiment was repeated in duplicate, with 2 mg. NaCN dissolved in 0.5 cc. of water (shown by the first experiment to be the approximate optimum moisture). The results are given in table 4.

TABLE 4

Sodium cyanide treatment (at 18°C.)

	A—LARVÆ	B—LARVÆ
1	None out	Dead
2	Dead	Dead
3	Dead	Dead
Check (no NaCN)	Very active	Slightly active

On crushing the gall in the cylinder 1A, the larvæ were found to be dead. This test shows conclusively the high killing effect of cyanide on the larvæ. This strength of cyanide (25 per cent) did not affect the eggs.

Sphagnum Moss

It has been noticed that roots of plants growing in sphagnum moss did not become infested with nematodes while roots on the plants not in sphagnum moss became infested.

This interesting point was summarily tested. Dry sphagnum moss was placed in a beaker of distilled water and boiled for 10 minutes. The mixture was then filtered and the moss extract applied at the rate of 0.5 cc. to gardenia galls in stender dishes as in previous experiments. The observations showed that the moss extract killed the larvæ (table 5).

TABLE 5

Effect of sphagnum moss extract treatment (at 18°C.)

	cc.	A	B
1	0.5	Dead	Dead
2	0.5	Dead	Dead
3	0.5	Dead	Dead
Check		Very active	Very active

Formaldehyde

A similar experiment was also tried with 40 per cent formaldehyde and the results are given in table 6.

This demonstrates the high killing effect of formaldehyde.

TABLE 6

Effects of formaldehyde (40 per cent) treatment (at 18°C.)

	cc.	A—LARVÆ	B—LARVÆ
1	0.5	Dead	Dead
2	0.5	Dead	Dead
3	0.5	Dead	Dead
Check		Very active	Very active

DISCUSSION OF RESULTS

The root-nematode (*Heterodera radicicola*) has been a serious pest to plants growing in certain soils and particularly in greenhouse soils. The investigations concerning this pest have been mainly morphological and the attempts to control it have thus far not given entirely satisfactory results. Steam sterilization of the soil does give complete control where it can be practically applied and with thoroughness, but the average conditions do not offer a way for the application. For example, greenhouses fitted for hot-water heating are not adapted to the use of steam. It was for such a condition that the attempt was made to find another method.

The first experiment was to determine the effect of moisture upon the nematode and it was observed that moisture is a necessity for activity but if the water content is in excess, then the animal becomes inactive. So there is an optimum moisture for the nematode.

An experiment to test the effect of temperature demonstrated that the nematode is inactive at comparatively low temperatures and at high temperatures, and its greatest activity is at a temperature from 65° to 85° F. So there is also an optimum temperature.

The recent success with sodium cyanide suggested this treatment as a possible remedy and it was found to be very effective in killing the larvæ and in a moderately concentrated solution to destroy the egg stage.

Similarly sphagnum moss extract and formaldehyde were found to be toxic to the nematode.

GREENHOUSE EXPERIMENTS

The satisfactory results of different treatments in the laboratory led to experiments with soil in the greenhouse. Fortunately, a very thoroughly infested greenhouse bench offered itself for the experiment. This bench had just produced tomato plants which were thoroughly infested with nematodes.

The tomato plants were removed and the soil raked clean of the roots and other objectionable materials and turned over thoroughly. Next the bench was divided into 7 plots, each separated by 5 inches of air space to prevent migration of nematodes from one plot to another.

Plots 2, 4 and 7 were used as check plots. Plots 1 and 3 were treated with sodium cyanide (NaCN) at the rates of 100 pounds and 200 pounds per acre (or 0.036 ounce and 0.072 ounce, respectively). The cyanide was dissolved in water at the rate of 1 gallon of water to $\frac{1}{2\frac{1}{8}}$ ounce and $\frac{1}{1\frac{1}{4}}$ ounce of cyanide, respectively.

The solution was applied to the soil with a sprinkling can at the rate of $\frac{1}{3}$ gallon per square foot of soil, and at the same time the soil was turned back and then thrown over to cover the solution. In this way every particle of soil received the same strength of the solution. After 4 days, the soil was broken up and aerated.

Another similar application was made to these two plots 5 days after the first application. This was done on the theory that the eggs of the animal might have resisted the first application and the larvæ would have hatched at the end of 5 days under the greenhouse temperature (70° to 80° F.) and would be killed by the second application of cyanide.

At the end of 3 days these soils were turned over and completely aerated to remove all traces of cyanide gas. The soil in these and check plots was all the time kept moist and in good tilth. At the end of another 3 days (February 7, 1917) these two plots and the check plots, No. 2 and 4, were planted to cucumbers, radishes and tomatoes. Mice attacked the seeds and necessitated replanting the cucumbers and radish seeds.

At this time it was thought that the mice might have carried some particles of soil from the infested check plots, so note was made of this partly to explain any error that might occur.

Plots 5 and 6 were treated with sodium cyanide at the rates of 50 and 100 pounds, respectively, to ascertain whether a weaker application would control the nematode, as labbratory experiments seemed to show, and whether or not one treatment would be sufficient. The same method of cyanide application was used as with plots 1 and 3. Plot 7 was reserved as a check plot. These plots were aerated 3 days after and planted 6 days after the application to lettuce, radishes, and tomatoes.

The percentage of seed germination was good and the seedlings grew well for about 3 weeks, when it became evident that they were not making average growth. Then it was realized that the soil was low in available nitrogen and tankage was subsequently applied at the rate of 10 gm. per square foot (or approximately 800 pounds per acre). In a few days the plants began to show the effect and made a rapid growth.

While waiting for the plants in these plots to make sufficient growth to observe results, several other experiments were performed.

The question of the effect of the application of sodium cyanide on plants growing in soil was tested by potting up 4 infested tomato plants in infested soil and allowing them to grow under normal conditions for 2 weeks. One of these plants was treated with cyanide dissolved in water and applied at the rate of 50 pounds per acre and another at 100 pounds per acre, which were amounts found to kill the nematode in the laboratory. The remaining two plots were used as checks. The plants in the treated pots wilted at the crown at the end of one day, and at the end of 5 days were completely dead, while the check plants grew as normally. Galls were examined from all plants and the nematode larvæ in them were found to be alive and very active.

The results of the greenhouse experiments were recorded from the time the plants had made a growth of 6 inches up to the time when they were 10 inches high (April 21, 1917). The plants were 18 weeks old at the time the final observations were recorded (table 7).

TABLE 7

Greenhouse experiments with cyanide treatment

DATE	NUMBER OF PLANTS EXAMINED	Plot 1			Plot 2			Plot 3			Plot 4			Plot 5			Plot 6			Plot 7		
		Galls			Galls			Galls			Galls			Galls			Galls			Galls		
		S*	M	L	S	M	L	S	M	L	S	M	L	S	M	L	S	M	L	S	M	L
March 31, 1917..	20	56	9	0	148	10	0	0	0	0	101	15	0	8	3	0	17	0	0	125	7	0
April 11, 1917....	18	30	9	1	130	9	7	2	0	0	40	0	0	15	0	0	60	2	0	37	0	0
April 18, 1917....	15	35	7	0	100	3	4	1	0	0	36	0	0	19	0	0	130	3	0	110	0	0
April 21, 1917....	27	30	3	10	120	7	13	0	0	0	57	2	2	30	0	0	110	3	2	130	10	15

*S = small M = medium L = large

This experiment demonstrated that cyanide to be effective must be applied in larger quantities than 100 pounds per acre to kill the larvæ, and under such applications the soil must be free from growing plants. As shown by the previous experiment, seed will germinate and plants will grow well in soil which has been treated with sodium cyanide used as high as 200 pounds per acre in two applications, and which has been well aerated after the application.

The table of observations shows that the treatment of 200 pounds of cyanide per acre followed by a second treatment 5 days later gives nearly a perfect control. Out of 80 plants examined only 3 galls were found. This is plainly

within experimental error and the results warrant the recommendation of this method of sterilization.

Due to the fact that the treated and untreated plots were so close together, some of the particles of soil and water carrying the nematodes could easily have been transmitted from one plot to the other.

There was no apparent difference in growth of plants in the various plots. They all were very healthy-looking at 18 weeks after the seed was planted. The experiment was then discontinued.

SUMMARY

1. A serious pest to plants, growing in warm soils and especially in greenhouse soils, is the root-nematode (*Heterodera radicicola*) sometimes called the eel-worm or root-gallworm.

2. This parasite is a minute organism which penetrates the roots of plants causing them to become enlarged and deformed, and resulting in a hindrance to growth.

3. The nematodes may be distributed by their own motion, by water, in soil clinging to implements, by infested plants transferred to an uninfested soil, by seed, by cuttings, and by manure.

4. The following conditions are necessary for their growth and reproduction: (a) an optimum moisture, (b) an optimum temperature, (c) food, and (d) oxygen.

5. The following were found to decrease nematode activity and may be applied as methods of control: (a) excess of moisture, (b) high temperature (101°F.), (c) formaldehyde, (d) sphagnum moss extract, and (e) sodium cyanide (NaCN) dissolved in water.

6. In experiments with greenhouse soils the following treatment gave a satisfactory and practical remedy for nematode infestation:

Sodium cyanide dissolved in water is applied in the proportion of 200 pounds per acre, one-third gallon per square foot of soil. One week after the first treatment a second similar treatment is given. This method is for soils free from plants. The soil should be aerated and leached well before seed or plants are put into it in order to remove the greater amount of cyanide gas.

7. The cost of materials for this treatment is $112 per acre. For greenhouse soils where steam sterilization is impracticable this is a remedy which is to be recommended.

CONCLUSIONS

The object of our study is thus accomplished. A satisfactory and practical control for the soil nematode is found in sodium cyanide.

In treating nematode-infested soil, the number of square feet of soil should first be determined. The number of pounds of cyanide should then be calculated on the basis of 200 pounds per acre. Next, the amount of water,

figured on the basis of ⅓ gallon per square foot of soil, should be determined. Dissolve the cyanide in the proper amount of water and apply the solution to the soil with a sprinkler. The soil should be well mixed and kept slightly moist and warm for 5 or 6 days before the treatment in order to bring the larvæ from their cysts and therefore render them more susceptible to the treatment. The third day after the first treatment the soil should be stirred and aerated slightly. The same favorable conditions for nematode activity should be maintained until after the second treatment. About one week after the first treatment the second application determined in the same manner should be given.

Of course, all plants should be removed from the soil before the application of cyanide, for most plants succumb to such a treatment. Before any seeds are planted or plants set in the sterilized soil, the soil should be thoroughly aerated and leached somewhat to remove the traces of cyanide gas.

Using this method of soil sterilization, the cost of the cyanide used would be 400 pounds at $.28 per pound, or $112. For greenhouse soil sterilization, this is an economical and entirely staisfactory investment.

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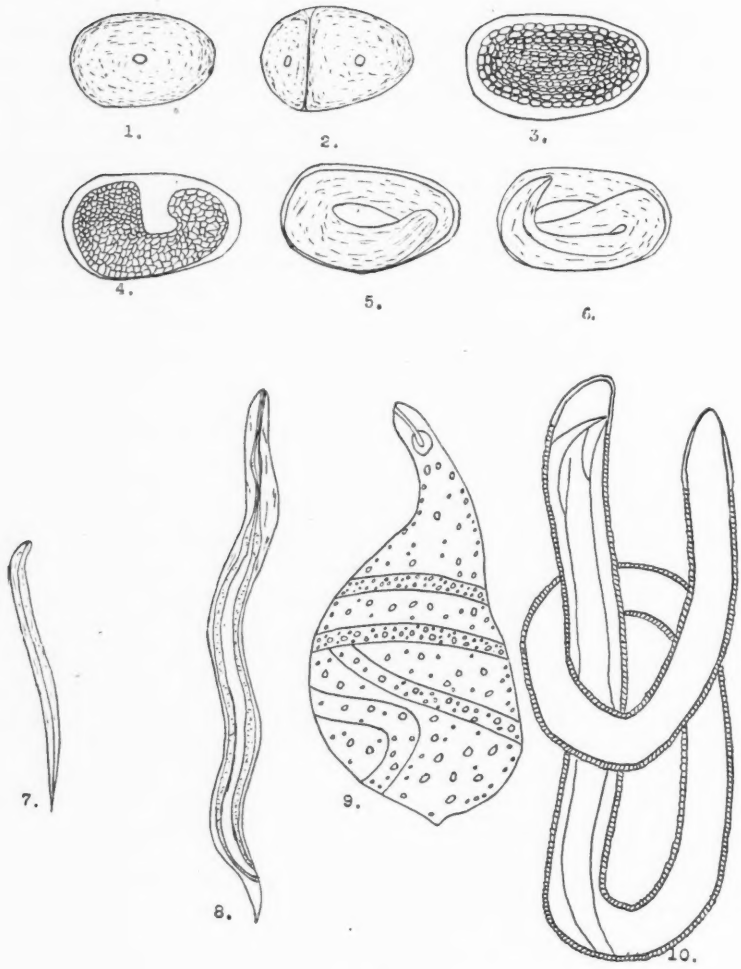
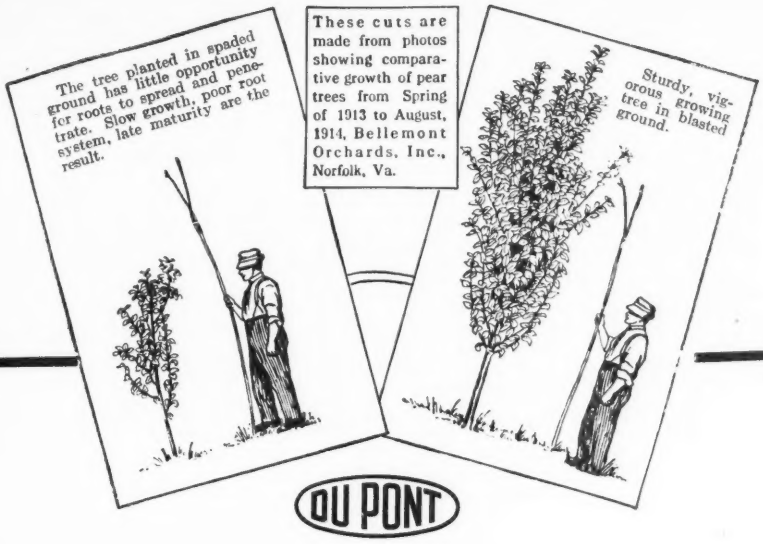


PLATE 1

FIGS. 1 to 6. DEVELOPMENT OF FREE-LIVING NEMATODE
FIG. 7. YOUNG LARVA
FIG. 8. IMMATURE FEMALE
FIG. 9. MATURE FEMALE
FIG. 10. MATURE MALE

INDEX



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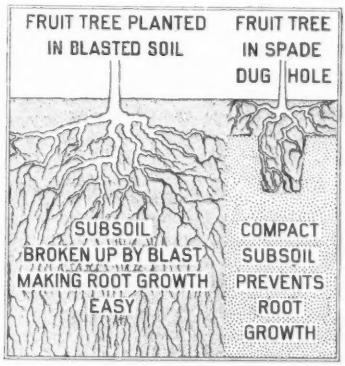

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CONTENTS

Inter woven

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TOE AND HEEL

Socks

NO EQUAL FOR WEAR

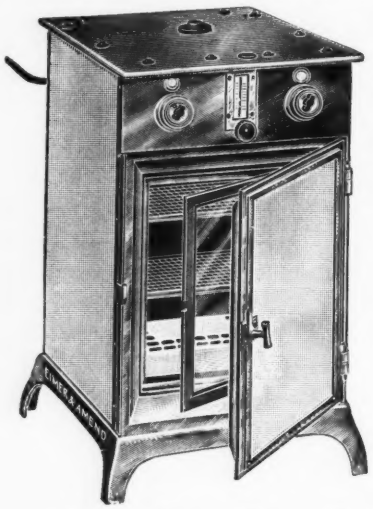
The Freas' Electric Incubator

Approved by the National Board of Fire Underwriters

Universally recognized as the most accurate and durable incubator available, and the only type that can be depended upon for continuous unattended operation. Made in six sizes.

The undermentioned advantages of the Freas' Electric Incubator strongly recommend its use—where electricity is available—in preference to any other incubator.

1. The almost perfect insulation against loss of heat by radiation cuts down the current consumption to about one-half that required for other forms. This implies less heating up of the laboratory, less strain on the wiring, less danger of sparking, and in general longer and more satisfactory service.



2. The "all-metal" regulating device maintains constant temperature indefinitely to a fraction of a degree.

3. To set for the temperature desired requires but a few moments of time. This is accomplished by simply turning a milled-head screw, which moves the indicator up or down the graduated temperature scale.

   Contrast this with the time required to set a mercury or other regulator of the most advanced type; the continuous watching required on account of the uncertainty of its proper and accurate regulation.

4. There are no complicated devices for heating, regulation, etc. The rugged construction of all parts of the Freas' Incubator is such that, with ordinary care, no parts are liable to get out of order.

5. Water is not required, nor employed, to maintain an even temperature throughout the chamber, thereby eliminating leakage, a disadvantage so common with copper incubators. The specifications explain the reasons for this advantage of the Freas' Incubator.

6. No auxiliary devices are needed. To operate, it is only necessary to screw the plug into a lamp socket, and turn on the current. The incubator will operate on either direct or alternating current. *When ordering specify the voltage.*

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